The effects of simulated wastewater nutrient amendments on *Sphagnum* productivity and decomposition within a subarctic ribbed fen[1]

by

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Abstract

Peatlands dominate the flat landscape of the Hudson Bay Lowland (HBL). *Sphagnum* mosses are the key peat-generating plants allowing for important ecosystem services such as carbon storage, climate regulation, and water polishing. The HBL is a location for current and proposed industrial mining development projects, and its peatlands may become increasingly used to polish treated wastewater from mining camps. This study focuses on biological changes in the *Sphagnum* moss community associated with the addition of simulated treated domestic wastewater to a subarctic ribbed fen (a wetland type commonly found throughout the HBL). We determined how the nutrient additions affected the productivity, decomposition, and nutrient ratios, within the ponds and raised peatland ridge components of the ribbed fen. Field experiment results show between a four to twelvefold increase in productivity rates of the low-lying *Sphagnum rubellum* species, and a twofold increase in productivity for the higher hummock or ridge dominating species *Sphagnum fuscum* in locations closest to the point source of nutrient effluent. Regions of the experimental ribbed fen greater than 50 m away from the point source showed little difference in productivity rates, nutrient content, or decomposition rate than the reference fen levels. No significant changes to the rate of decomposition of *Sphagnum* were observed with relation to distance away from point source nutrients as the experimental fen decomposition rates were comparable to the reference fen rates. A laboratory peat incubation experiment was conducted to determine how increasing exposure to the wastewater nutrients would affect *Sphagnum* decay potentials. Lab results indicate that greater concentrations of nutrient additions to incubation environments did not significantly increase the amount of CO_2 or CH_4 emissions. However, origin of the peat and the species of *Sphagnum* moss comprising the peat was found to be important factors contributing to *Sphagnum* decomposability and greenhouse gas emissions. Peat formed within a nutrient enriched location produced significantly greater CO_2 and CH_4 emissions than peat originating from non-fertilized locations, and hollow dominant *Sphagnum* species show greater decomposability than hummock forming species.

makers and industries will consult these results for mining development projects within the HBL and elsewhere in subarctic and boreal biomes.

Keywords: Ribbed fen, subarctic peatland, Hudson Bay Lowlands, *Sphagnum* moss, carbon storage, treatment wetlands

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Preface

Peatlands store large amounts of terrestrial carbon because the rate of primary productivity exceeds the rate of organic matter decay. They cover approximately 3% of the Earth's land area and store 15 to 30 % of global soil carbon, especially in boreal and subarctic landscapes (Limpens *et al*. 2008; Yu *et al*. 2010). These northern peatlands include three of the ten largest wetlands in the world (Keddy *et al.* 2009). The Hudson Bay Lowland (HBL) is the third largest in area, spanning 373,700 km^2 across north-central Canada, but it is perhaps the most carbon-dense (Abraham and Keddy 2005; Gorham 2003). The key peat-forming vegetation contributing to the accumulation of peat on the landscape is *Sphagnum* moss (Abraham and Keddy 2005), which has been identified as the keystone genus of the north because of its inherent resistance to decay and its survival in cool climate, nutrient poor conditions (Rochefort 2000). Current and projected changes in northern climate, especially around Hudson and James Bays (McLaughlin and Webster 2013) place an increased spotlight on this carbon sequestration service of the peatlands of the HBL, especially the contribution of *Sphagnum*.

Peatlands provide an array of regulating ecosystem services in addition to the sequestration and storing carbon, such as erosion protection, water quantity regulation and water filtration and purification (Kimmel and Mander 2010). Humans have commonly used the filtering function of wetlands ecosystems such as marshes to polish secondarily-treated domestic wastewater (Kadlec and Wallace 2009), but we have less often harnessed northern peatlands to polish wastewater (Kadlec 2009; Ronkanen and Klove 2009). This wetland polishing is a tertiary treatment which is meant to remove removes excess nutrients and other contaminants.

The HBL peatlands are currently considered pristine due to their lack of large-scale human or industrial developments, however, large deposits of mineral resources have been discovered in

the area and an increase in industrial and human developments may arise within the coming decades (Far North Science Advisory Panel 2010). The increasing numbers of remote mining camp operations, as well as an increasing population within indigenous communities would result in a greater need for the HBL peatlands to perform wastewater polishing services. Few studies have assessed the ability of Canadian subarctic peatlands to polish treated domestic wastewater. There is a lack of understanding on the biological and hydrological response of peatlands within northern subarctic climates, such as the HBL, to cope with high nutrient loading at a point source resulting from anthropogenic developments.

In a collaborative study, McCarter and Price (2017), and McCarter *et al.* (2017) provided insight on the hydrological response and nutrient transport, while Twible and Branfireun (in preparation) provide insight on the biogeochemical response of a subarctic ribbed fen receiving simulated secondarily-treated domestic wastewater from a remote mining camp operation. This current thesis aims to complement the previous and on-going research by providing insight into the biological response to the point source nutrient loading by assessing the impact on the rates of productivity and decomposition of key *Sphagnum* species and the nutrient content and C:N ratios within *Sphagnum* tissues.

My thesis is composed of two research chapters, each written in manuscript form. Chapter 1 focuses on (i) measuring the field rates of *Sphagnum* productivity and decomposition, as well as (ii) determining nutrient concentrations within the *Sphagnum* tissues themselves across the hydrological and nutrient gradient of the experimental fen, as compared to a non-fertilized reference fen. Chapter 2 is a laboratory peat incubation experiment that focuses on determining the microbial decomposition (CO_2 and CH_4 emissions) of three species of *Sphagnum* mosses exposed to increasing concentrations of nutrient loading. Both chapters work towards

understanding how nutrient additions associated with treated domestic wastewater would impact *Sphagnum*-peat formation and decomposition. Shifts to the rates of *Sphagnum* productivity and decay would directly impact the quantity and quality of carbon storage, the hydrology and the wastewater polishing services provides by the HBL peatlands.

I am submitting my Master of Science thesis in the form of two manuscripts. Chapter 1 is a manuscript co-authored by my thesis supervisor, Daniel Campbell, Jim McLaughlin from the Ontario Forestry Research Institute and myself. Chapter 2 is a manuscript co-authored by Daniel Campbell and myself. For both chapters, I assisted in planning the experiments along with Daniel Campbell, and I was responsible for conducting these experiments in the field and in the lab. I was in charge of sampling, data analysis, and writing. Daniel Campbell contributed to the experimental design and planning of both experiments, and helped determine which parameters to focus on and was also involved with brainstorming the statistical analysis and editing my writing. Given my role in these manuscripts, I am submitting them as my Master of Science thesis.

Literature Cited

Abraham, K.F. & Keddy C.J. (2005). The Hudson Bay Lowland. *In* L.H. Fraser and P.A. Keddy (eds.). *The world's largest wetlands: Ecology and conservation.* Cambridge, UK: Cambridge University Press. pp 118-148.

Far North Science Advisory Panel. (2010). Science for a Changing Far North. The Report of the Far North Science Advisory Panel. A report submitted to the Ontario Ministry of Natural Resources.

Gorham, E., Janssens, J., & Glaser, P. (2003). Rates of peat accumulation during the postglacial period in 32 states from Alaska to Newfoundland, with special emphasis on northern Minnesota. *Canadian Journal of Botany,* **81**, 429-438.

Kadlec, R. H., & Wallace, S. D. (2009). Treatment Wetlands (2nd ed.). Boca Raton, FL: CRC Press, Taylor & Francis Group.

Kadlec, R. H. (2009). Wastewater treatment at the Houghton Lake wetland: Hydrology and water quality. *Ecological Engineering,* **35**, 1287–1311. doi:10.1016/j.ecoleng.2008.10.001

Keddy, P. A., L. H. Fraser, A. I. Solomeshch, W. J. Junk, D. R. Campbell, M. T. K. Arroyo, & C. J. R. Alho. (2009). Wet and wonderful: the world's largest wetlands are conservation priorities, *BioScience*, **59**(1), 39-51.

Kimmel, K., & Mander, Ü. (2010). Ecosystem services of peatlands: Implications for restoration. *Progress in Physical Geography,* **34**(4), 491-514.

Limpens, J., Berendse, F., Blodau, C., Canadell, J. G., Freeman, C., Holden, J., ... & Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: from local processes to global implications–a synthesis. *Biogeosciences,* **5**(5), 1475-1491.

McCarter, C. P. R., Branfireun, B. A., & Price, J. S. (2017). Nutrient and mercury transport in a sub-arctic ladder fen peatland subjected to simulated wastewater discharges. *Science of The Total Environment*, ***609***, 1349-1360.

McCarter, C. P., & Price, J. S. (2017). The transport dynamics of chloride and sodium in a ladder fen during a continuous wastewater polishing experiment. *Journal of Hydrology*, **549**, 558-570.

McLaughlin, J., & Webster, K. (2013). Effects of a changing climate on peatlands in permafrost zones: a literature review and application to Ontario's Far North (No. CCRR-34). Ontario Forest Research Institute.

Rochefort, L. (2000). Sphagnum—a keystone genus in habitat restoration. *The Bryologist*, **103**(3), 503-508.

Ronkanen, A.-K., and Klove, B. 2009. Long-term phosphorus and nitrogen removal processes and preferential flow paths in northern constructed peatlands. *Ecological Engineering,* **35(5),** 843-855 doi:10.1016/j.ecoleng.2008.12.007.

Yu, Z., J. Loisel, D. P. Brosseau, D. W. Beilman, & S. J. Hunt. (2010). Global peatland dynamics since the Last Glacial Maximum. *Geophys. Res. Lett.,* **37**, L13402, doi:10.1029/2010GL043584.

CHAPTER 1: The effects of simulated treated domestic wastewater on *Sphagnum* productivity, decomposition, and nutrient dynamics in a subarctic ribbed fen

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Abstract

Peatlands dominate the flat landscape of the Hudson Bay Lowland (HBL). *Sphagnum* mosses are the key peat-generating plants allowing for important ecosystem services such as carbon storage and water polishing. The HBL also has current and proposed industrial mining development projects, and its peatlands may become increasingly used to polish secondarily-treated wastewater from mining camps. We examined biological changes in the plant community associated with the addition of simulated secondarily-treated wastewater to a subarctic ribbed fen, a wetland type commonly found throughout the HBL. We determined how the nutrient additions affected the productivity, decomposition, and nutrient ratios within the ponds and raised peatland ridge components of the ribbed fen. Our results show between a four to twelvefold increase in productivity rates of the low-lying *Sphagnum rubellum* species, and a twofold increase in productivity for the higher hummock or ridge-dominating species *Sphagnum fuscum* in locations closest to the point source of nutrient effluent. Regions of the experimental ribbed fen greater than 50 m away from the point source showed little difference in productivity rates or nutrient content than the reference fen levels. No significant changes to the rate of decomposition of *Sphagnum* were observed with relation to distance away from point source nutrients as the experimental fen decomposition rates were comparable to the reference fen rates. *Sphagnum* productivity per year remained greater than mass lost to decomposition. Therefore, this study suggests that, in the short-term, subarctic peatlands exposed to nutrient levels comparable to that present in treated domestic wastewater will increase their capacity to generate *Sphagnum*-peat and store carbon. This experimental research aids in understanding to what degree plants mediate shifts in ecosystem dynamics within subarctic ribbed fens. Policy makers,

community planners, and industries may consult these results for mining development projects within the HBL and elsewhere in subarctic and boreal biomes.

Keywords: Subarctic peatland, ridge-pool sequence, Hudson Bay Lowland, *Sphagnum* moss, nutrient enrichment, tertiary treatment, wastewater polishing, remote resource development, mining

Data repository: Scholars Portal Dataverse

Introduction

Peatlands have a rate of primary production which exceeds their rate of decomposition, so they sequester and store carbon as peat. They cover approximately 3% of the Earth's land area and store 15 to 30 % of global soil carbon, especially in boreal and subarctic landscapes (Limpens *et al*., 2008; Yu *et al*., 2010). These northern peatlands remain largely pristine (Potapov *et al.*, 2008; Lee *et al.*, 2010) and include three of the ten largest wetlands in the world (Keddy *et al.*, 2009). The Hudson Bay Lowland (HBL) is the third largest wetland in area, spanning 373,700 km^2 across north-central Canada, but it is perhaps the most carbon-dense (Gorham, 1991; Abraham and Keddy, 2005). Peatlands provide an array of regulating ecosystem services in addition to the sequestration and storing carbon, such as erosion protection, water quantity regulation and water filtration and purification (Kimmel and Mander, 2010). Humans have commonly used the filtering function of wetlands ecosystems such as marshes to polish secondarily-treated domestic wastewaters (Kadlec and Wallace, 2009), but we have less often harnessed northern peatlands to polish wastewater (Kadlec, 2009; Ronkanen and Klove, 2009, McCarter *et al.*, 2017).

Northern peatlands are dominated by ombrotrophic bogs and ribbed fens (Keddy *et al.*, 2009; Riley, 2011). Bogs only receive water from precipitation and serve as water storage complexes, whereas fens act as conveyors of water from peatlands across the landscape during periods of high hydrological connectivity (Quinton *et al.*, 2003). Ribbed fens have a repeating pattern of pool-ridge-pool morphology with slow hydrological flow running perpendicular to the raised peat ridges (Price and Maloney, 1994; Quinton and Roulet, 1998). Hydrological connectivity within a ribbed-fen occurs by a spill-and-fill mechanism when the water table rises above the height of the peat ridge (Spence and Woo, 2003), and by pool-to-pool connectivity via

low-lying preferential flow paths (Price and Maloney, 1994; Quinton and Roulet, 1998). Such fen complexes with gentle slopes and hydrological flow are better suited for water polishing services (McCarter, 2016).

Both ombrotrophic bogs and ribbed fens are acidic, nutrient-deprived ecosystems dominated by *Sphagnum* mosses (Riley, 2011; Sjörs, 1963). Their rates of productivity and decomposition depend on the climate, degree of waterlogging, the nutrient composition and nutrient availability within the peatland (Clymo and Hayward, 1982) as well as the dominant *Sphagnum* species (Heal *et al*., 1978; Clymo, 1984). Both bogs and fens produce hummocks and hollows, with intermediate carpets, each with its own assemblages of *Sphagnum* species (Andrus *et al*., 1983). Hummock species, such as *S. fuscum*, have slower growth but are more recalcitrant to decomposition as compared to hollow species (Clymo and Hayward, 1982; Johnson and Damman, 1991; Turetsky *et al*., 2008). This recalcitrance to decay makes *Sphagnum* species the predominant peat-formers in northern peatlands. Their peat forms the ridges in ribbed fens, so their growth and decomposition in turn controls the hydrology of these peatland landscapes.

Sphagnum species are acid-generating (Clymo, 1963) and have a high cation-exchange capacity relative to other plant life-forms, allowing them to efficiently scavenge for nutrients in these nutrient-poor environments (Clymo and Hayward, 1982). However, nutrient enrichment can change *Sphagnum* growth and decomposition and potentially hydrological relations in peatlands, as has been found in numerous studies simulating enriched atmospheric N deposition. Early European studies reported decreases in *Sphagnum* growth rates with enriched atmospheric N deposition, and in some cases, *Sphagnum* mortality (Ferguson *et al*., 1984; Press *et al*., 1986; Woodin and Lee, 1987; Verhoeven and Schmitz, 1991). Aerts *et al*., (1992) conducted a fertilization experiment in European ombrotrophic bogs to simulate atmospheric N-enrichment

and found that the addition of N to peatlands with low atmospheric N deposition caused a fourfold increase to the growth rate of *Sphagnum.* Those peatlands exposed to higher atmospheric N deposition (>4 g m^{-2} $year^{-1}$) were no longer N-limiting, but become P-limiting. Other European peatland fertilization experiments have generally determined that high rates of atmospheric N deposition cause a decrease in *Sphagnum* biomass and peat formation (Berendse *et al.*, 2001; Van Wijk *et al.*, 2003). In North America, Bubier *et al*., (2007) conducted a five-year nutrient addition experiment to an ombrotrophic bog in eastern Ontario using treatments representative of elevated atmospheric N deposition (1.6 to 6.4 g m^{-2} yr^{-1}), and some treatments also added P (5 g m^{-2} yr^{-1}) and K (6.3 g m^{-2} yr^{-1}). Over the first two years, plant growth and net ecosystem CO_2 exchange (NEE) increased, indicating that the bog increased its carbon storage capacity. By the third year and onward NEE levels began to decrease, as the plant community shifted away from *Sphagnum* toward other mosses and vascular plants. After four years of nutrient loading, the treatments with greater N loading, as well as P and K, had no more *Sphagnum* cover because of competition for light with taller vascular plants such as shrubs (Bubier *et al*., 2007). Larmola *et al*., (2013) concluded that the vegetation shifts, particularly the loss of *Sphagnum*, is the key explanation why peatlands with longer-term nutrient enrichment become weaker carbon sinks. However, most of these studies have focused on atmospheric enrichment with low input rates, in ombrotrophic peatlands where the water is stagnant, and often under warmer climates and greater anthropogenic influence as compared to the majority of peatlands in boreal and subarctic landscapes. Limited research has been conducted in fens or with higher enrichment rates or with point source inputs.

Kadlec (2009) conducted detailed studies of a wastewater polishing peatland running from 1970 to 2010 in Houghton Lake, Michigan. The municipality discharged point source additions

of wastewater into a 700 ha peatland seasonally (May-October) at a loading rate of 600,000 m^3 $year^{-1}$ with concentrations of 7.5 mg/L of dissolved inorganic nitrogen and 3.5 mg/L total phosphorus (Kadlec, 2009). These enrichment levels far exceed that of the other peatland fertilization studies focused on nutrient loadings simulating potential atmospheric deposition. Within the first few years of wastewater discharge changes to vegetation composition were significant, as *Typha* spp. began to increase in abundance and density to the point of invasion and mass displacement of original peatland plant assemblages (Kadlec and Bevis, 2009). Peat structure shifted from predominantly *Sphagnum* and sedge peat to floating mats of *Typha* spp. with far faster rates of decomposition (Kadlec and Bevis, 2009).

Resource development pressures and the population growth of isolated communities have increased in remote northern regions, such as in the HBL (Far North Science Advisory Panel, 2010). In consequence, peatland systems may be increasingly used to polish secondarily-treated wastewater. There is a lack of understanding on the biological and hydrological response of peatlands within northern subarctic climates, such as the HBL, to cope with high nutrient loading at a point source resulting from anthropogenic developments.

In a collaborative study, McCarter (2016) provided insight on the hydrological response of a subarctic ribbed fen receiving simulated secondarily-treated domestic wastewater from a remote mining camp operation. This current study aims to provide insight on the biological response to the point source nutrient loading by assessing the impact on the rates of productivity and decomposition with key *Sphagnum* species, and the nutrient content and C:N ratios within *Sphagnum* tissues. We hypothesized that the addition of simulated treated domestic wastewater will, in the short-term, (i) increase the rate of productivity of peat-forming *Sphagnum* species; (ii) increase the rate of decomposition of the peat; and (iii) increase nutrient content and decrease

C:N ratios of *Sphagnum* tissues. We also hypothesized that the production, nutrient uptake and decomposition of hollow *Sphagnum* species would be more influenced by the nutrient additions than hummock *Sphagnum* species, since they are at or near the water table.

Methods

We conducted the study near the De Beers Canada Victor Mine, within the Attawapiskat River watershed of the Hudson Bay Lowland (HBL) in north-central Canada (52°49'08" N, 83°54'52" W; 80 m elevation). The HBL is a vast peatland plain underlain by glaciomarine sediments and limestone bedrock (Martini, 2006). It is the world's third largest peatland, spanning 373 700 km^2 (Abraham and Keddy, 2005), with average peat depths between 1-3 m (Riley, 2011), making it a globally significant carbon sink with estimated storage of 20-30 g C m^{-2} $year^{-1}$ (Gorham *et al.*, 2003). Hudson Bay and James Bay heavily influence this region, creating a cool, humid, high-boreal climate, characterized by short cool summers and long cold winters (Abraham and Keddy, 2005: Riley, 2011). The mean annual temperature is -1.3 °C, with a mean of -22.3 °C in January, and 17.2 °C in July (Lansdowne House; 52°14' N, 87° 53' W; 280 Km WSW; 254 m elevation; 1971-2000 normals; Environment Canada, 2016). The mean annual precipitation is 700 mm, with 291 mm falling during from June to the end of August. At the Victor Mine, the 2015 growing season was wet, with 364 mm of precipitation from June 1 to August 31, 2015, but only 157 mm during the same time period in 2016 (De Beers Canada, unpublished data).

We studied two ribbed fens: an experimental fen (52°51'17" N, 83°56'35" W) and a reference fen (52°47'00" N, 83°53'21" W; Figure 1). The fens are approximately 8.5 km apart and drain into separate tributaries of the Attawapiskat River. The experimental fen has a mean

peat depth of 2.05 m, a total area of 9800 m^2 (2240 m^2 pools, 7560 m^2 ridges), and an elevation drop of 0.67 m across its 250 m length (McCarter, 2016). Its vegetation is split into two distinct zones: a poor fen with distinct pool-ridge morphology (0-140 m), and a richer fen dominated by carpets of *Sphagnum rubellum* and minimal ridge-pool morphology (140- 200 m; McCarter, 2016). The reference fen had similar peat depth and drop in elevation over its 150 m length, with a distinct pool-ridge morphology throughout, and poor fen vegetation (McCarter, 2016).

McCarter (2016) added simulated domestic wastewater additions in 2014 and 2015. Briefly, the experimental fen received a continual input of simulated wastewater from a point source for 51 days in the summer 2014 and 41 days in summer 2015 at a rate of 38 m^3 day^{-1}. The simulated wastewater was specially formulated to mimic the secondarily-treated wastewater effluent from the Victor Mine camp or a small isolated community in northwestern Ontario (McCarter, 2016), and contained SO_4^{-2} (27.2 mg L^{-1}), NO_3^- (27.2 mg L^{-1}), NH_4^+(9.1 mg L^{-1}), PO_4^{-3} (7.4 mg L^{-1}), K^+ (24.5 mg L^{-1}). A sodium chloride salt tracer was also added to the experimental fen, so the simulated wastewater also contained Na^+ (25.3 mg L^{-1}) and Cl^- (47.2 mg L^{-1}).

We established 72 sampling points in the experimental fen and 18 in the reference fen in May 2015 using a stratified sampling design. Within the experimental fen, we first selected sample zones up-gradient from the effluent discharge point (pool 0 and ridge 0) and down-gradient, generally at each ridge and pool. Within each sampling zone, we selected three sampling sites within a ridge in hummocks of *Sphagnum fuscum*, three along the edge of the pools in carpets of *Sphagnum rubellum,* and three *Sphagnum majus* sampling sites within a pool. We evenly distributed our sampling sites within each zone, and selected only undisturbed, monospecific colonies. At each sampling site, we randomly chose three sample points. Within

the reference fen, we only sampled near the start and end of the fen, again with three sampling sites in either zone in *Sphagnum fuscum* ridges, *S. rubellum* carpets along pools, and the aquatic *S. majus* within the pools, with three randomly chosen points within each site.

To determine if there were temperature differences between microtopographical positions, we placed HOBO® Pendant temperature loggers at random ridge and pool sample sites throughout the experimental fen at a depth of 10 cm. Loggers recorded temperature every four hours throughout the 2016 12-week growing season.

We measured the productivity of *Sphagnum fuscum* and *S. rubellum* using the crank wire method (Clymo and Reddaway, 1974), with five replicate crank wires at each sampling point. Briefly, we made the crank wires with 0.81 mm diameter (20 gauge) stainless steel wire bent into a crank shape so that there was 10 cm of straight vertical wire on either side of a 1 cm long horizontal cranked section. We inserted each crank wire into a *Sphagnum* carpet so that the horizontal crank was level with the tops of the capitula and the remaining 10 cm of vertical wire was suspended in the air. We measured productivity by measuring the length from the top of the *Sphagnum* capitula to the top of the wire, using calipers. In 2015, we measured growth from July 23 to September 8 (6.5 weeks), and in 2016, from June 6 to September 2 (12 weeks). We separated the 2016 season into two ~6-week periods, from June 6 to July 27 and from July 28 to September 2, the second providing a comparable time fame to the 2015 productivity measurements.

We determined the decomposition rate of *S. fuscum* and *S. rubellum* by means of mass loss over time using the mesh decomposition bag technique (Johnson and Damman, 1991). Briefly, we harvested *Sphagnum* strands from each sample point in the experimental fen and reference fen in July 2015. We removed and discarded the top 1 cm of each strand including the capitulum,

and then cut each strand to 5 cm length and discarded the older, bottom portion of the strand to ensure a constant age of *Sphagnum* strands. We determined their initial dry mass by placing ten 5 cm strands in the drying oven at 30 °C for 48 hours prior to weighing. We then placed the ten strands in 0.2 mm diameter mesh nylon bags and heat-sealed them closed. We returned the decomposition bags with the *Sphagnum* strands to the exact same sampling points in the field from which they were harvested, with two replicate decomposition bags per sampling point, and buried them at a depth of 10 cm below the surface. We also dried a subsample of each group of *Sphagnum* strands at 70 °C for 48 hours to determine if there was any residual water and to calculate a conversion factor between 30 °C to 70 °C drying temperatures. One year later in July 2016, we retrieved the decomposition bags from the field, rinsed them with deionized water to remove external organic debris, and then oven dried them at 70 °C for 48 hours. We carefully separated the remaining *Sphagnum* strands from the mesh bags and weighed them. We calculated decomposition rates by correcting the initial mass at 30 °C using the calculated conversion factor, then subtracting the final mass at 70 °C from initial mass at 70 °C. We divided the mass loss decomposition data by each sampling location's unique *Sphagnum* carpet density value, which we previously determined by taking the mean number of *Sphagnum* strands per dm^2 area at each sampling zone.

To determine nutrient content within the *Sphagnum*, we collected samples of *S. fuscum*, *S. rubellum*, and *S. majus* from the sampling locations in the Experimental and Reference Fens in August of 2016. We removed the capitulum of each *Sphagnum* strand to have strictly non-living (litter) components of the moss tested for nutrient analysis, and to remain consistent with the procedures used in the growth and decomposition experiments. We air-dried samples for 30 days at room temperature, ground them to a fine power using a ball mill grinder (RETSCH® Mixer

Mill 400), placed them into clean dry 20 ml glass scintillation vials, and sent them to the Ontario Forestry Research Institute (OFRI) for nutrient analysis. They analyzed the samples for total concentrations of C and N using an elemental combustion analyzer (Vario MAX Cube CN), total S using a carbon/sulfur combustion analyzer (ELTRA® CS-800), and total P, K, Ca and Mg using inductively coupled plasma (ICP; Genesis FEE ICP OES) run off a selenium dioxide extraction following industrial method NO 786-86T (Bran and Luebbe, 1986).

We mapped the productivity and decomposition data of the experimental and reference fens using size proportional bubble maps created in ArcGIS (ArcMap10.0, ESRI 2011). To reduce the complexity of the nutrient data, we first conducted a principal component analyses of the macronutrient variables within the *Sphagnum* plants (TN, TS, P, K, Ca and Mg), using PRIMER® version 7. We used the first principal component (PC1) as well as the C:N ratio in subsequent analyses. We then used a three-step approach to analyze the productivity, decomposition and nutrient data. First, we conducted simple analyses of variance to determine differences among species across all sample points. Second, we analyzed the growth, decomposition, and nutrient data separately by species using a linear regression against distance downgradient, with the fen as a categorical variable and their interaction. In the experimental fen, we only included samples downgradient from the nutrient point source in the regression analyses. We performed these analyses with Statistica® version 10, using a type I error rate of 5%, although we considered effects noteworthy with up to a 10% error rate. Third, we explored for discontinuities in the shape of the individual regressions within the experimental fen to identify breakpoints using segmented regression with the software SegReg® (www.waterlog.info). Segmented regression models were only chosen if they were superior to the linear regression models, as determined from their type I error rate. We also performed

Pearson correlation tests on the growth, decomposition, and nutrient data using SPSS®, (version 21, 2012).

Results

Surface temperatures within both ribbed fens increase over time throughout the duration of the growing season (June to end of August) in both the 2015 and 2016 summers (Figure 2). In both the experimental and reference fens the pools are consistently slightly warmer in temperature than the ridges, by 2.5 to 3.5 ^{o}C over the growing season average, with median temperatures in August, the hottest month, of 16.4 ^{o}C in pools and 14.2 ^{o}C in ridges.

The rates of productivity for both *Sphagnum* species were strongly correlated among the 6-week and 12-week time periods in 2015 and 2016 ($r > 0.80$; Table 1). When we just consider the productivity of both species across all sampling site distances in both fens, *S. fuscum* did not differ in productivity from *S. rubellum* (2015 6-weeks: $F_{1,41} = 0.62$, $P = 0.44$; 2016 6-weeks: $F_{1,42} = 1.50$, $P = 0.23$; 2016 12-weeks: $F_{1,42} = 1.95$, $P = 0.17$).

When we look at each species individually, the productivity of *S. fuscum* within the experimental fen did not differ significantly from the reference fen over the 6-week periods in the 2015 and 2016 growing seasons (both $P = 0.79$; Table 2; Figures 3 and 4) or over the 12-week period in the 2016 growing season ($P = 0.97$; Table 2; Figures 5 and 6). The productivity of *S. fuscum* also did not show a linear regression with distance downgradient in either fen over any time period (Table 2), but when we examined for a segmented regression in the experimental fen, the productivity of *S. fuscum* was slightly higher at all time periods near the point source and dropped to until a common breakpoint of 48 m downgradient of the point source, after which there was no change (2015 6-weeks: $P = 0.065$; 2016 6-weeks: $P = 0.039$; 2016 12-weeks: $P = 0.024$; Figures 4 and 6).

In contrast, the productivity of *S. rubellum* within the experimental fen was significantly greater than the reference fen at all time periods (2015 6-weeks: $P = 0.014$, 2016 6-weeks: $P =$ 0.0.023, 2016 12-weeks: $P = 0.019$; Table 2; Figure 3 to 6). The productivity of *S. rubellum* did not show a linear regression with distance downgradient over any time period, although a borderline interaction occurred (Table 2), but when we examined for a segmented regression, the productivity of *S. rubellum* was much greater at all time periods near the point source of nutrient input until a common break point at 38 m from the point source (all $P < 0.0001$; Table 2), after which, there was no change as productivity (Figures 4 and 6).

The one-year decomposition for both *Sphagnum* species did not significantly correlate with any measures of productivity at any time (Table 1). Based on initial analysis of variance of the data among species, the decomposition rates did not differ between *S. fuscum* and *S. rubellum* ($F_{1,37} = 2.04$, $P = 0.16$). When we consider each species individually, the decomposition of *S. fuscum* within the experimental fen did not differ significantly from the reference fen ($P = 0.38$; Table 2; Figures 5 and 6). Its decomposition did not show a linear regression with distance downgradient ($P = 0.19$; Table 2), with no interaction, and we found no segmented regression in the experimental fen ($P = 0.78$). For *S. rubellum*, its decomposition was borderline higher in the experimental fen than in the reference fen ($P = 0.08$; Table 2; Figures 5 and 6), but its decomposition did not change with distance downgradient ($P = 0.35$), with no interaction (Table 2), and we found no segmented regression in the experimental fen ($P = 0.12$).

The first principal component (PC1) of nutrient content within the *Sphagnum* tissues explained 74% of the total variation, while the second component (PC2) only explained 17% of the variation (Figure 7). PC1 was strongly positively correlated with TN, TS, TP, K, and Mg, while PC2 was strongly but negatively correlated with Ca (Table 2). This PC1 variable was

moderately to strongly correlated to C:N ratio within each species (*S. fuscum*: $r = 0.53$; *S. rubellum*: $r = 0.81$; *S. majus*: $r = 0.83$).

All three *Sphagnum* species had significantly different nutrient contents in their tissues as determined by their PC1 values ($F_{2,65} = 37.75$, $P < 0.0001$, Figure 8A), with *S. fuscum* and *S. rubellum* differing from each other slightly ($P = 0.020$) and *S. majus* differing strongly from both these species ($P = 0.0001$). For *S. fuscum*, PC1 of plant nutrient content did not differ significantly between fens, there was no effect of distance, and no interaction (Table 3). *S. fuscum* also showed no segmented regressions of PC1 with distance within the experimental fen. Likewise, the C:N ratio of *S. fuscum* did not differ between fens, again with no regression against distance, no interaction (Table 3; Figure 8B) and no segmented regression.

For *S. rubellum*, PC1 of plant nutrient content was borderline higher in the experimental fen ($P = 0.072$), indicating more plant nutrient content in the experimental fen, but there was no effect of distance and no fen by distance interaction (Table 3; Figure 8A). *S. rubellum* also showed no segmented regression. C:N ratio of *S. rubellum* did not differ between fens, again had no regression with distance, no fen by distance interaction (Table 3), and no segmented regression (Figure 8B).

The PC1 of plant nutrient content for *S. majus* was much higher in the experimental fen than the reference fens ($P = 0.0002$; Table 3; Figure 8A), and there was a significant negative regression with distance downgradient ($P = 0.045$). A borderline significant fen by distance interaction ($P = 0.07$) shows that the negative regression with distance only occurred in the experimental fen (experimental: $P = 0.004$; reference: $P = 0.55$). PC1 showed no segmented regression, thus a simple linear regression fits the data best. The C:N ratio of *S. majus* was significantly lower in the experimental than in the reference fen ($P = 0.0002$; Table 3; Figure

8B). C:N ratio increased significantly with distance downgradient in both fens ($P = 0.045$), with no interaction, suggesting that both the experimental and reference fen show an increasing C:N trend with increasing distance downgradient (Table 3; Figure 8), however, when C:N ratios were analyzed separately for each fen, only the experimental fen showed significant regression with distance (experimental: $P < 0.0001$; reference $P = 0.55$). C:N ratio for *S. majus* also showed no segmented regression (Figure 8).

Within the experimental fen, *S. fuscum* PC1 and C:N ratios significantly correlated with the 2015 six-week productivity rates (PC1: $r = 0.559$, $P = 0.038$; C:N: $r = -0.69$, $P = 0.006$), but, did not significantly correlate with productivity during the 2016 season (Table 1). For *S. rubellum* in the experimental fen, only PC1 significantly correlated with 2015 6-week productivity rates ($r = 0.741$, $P = 0.004$; Table 1). PC1 and C:N ratios for *S. fuscum* or *S. rubellum* did not correlate with decomposition at any time (Table 1).

Discussion

We examined changes in *Sphagnum* productivity and decomposition rates following point source press applications of treated domestic wastewater, as well as compare uptake of nutrients across the hydrological flow gradient of the experimental fen.

We had first hypothesized that the point source addition of simulated treated wastewater would increase *Sphagnum* productivity within the experimental fen, and that the carpet species would show a greater productivity response to the nutrient additions than the hummock species. Our experimental results supported our hypothesis. The lower-lying *S. rubellum* showed significantly greater productivity rates within the experimental fen compared to reference fen levels, and *S. fuscum*, the higher hummock species, showed little to no growth response with

added nutrients and maintained similar productivity levels throughout both fens. These results support other research conducted on *Sphagnum* productivity rates in northern low-nutrient peatlands (Turetsky *et al*., 2008), and northern peatlands exposed to increased nutrient fertilization (Aerts *et al*., 1992; Aerts *et al*., 2001).

Soil moisture is a key environmental factor that influences *Sphagnum* productivity (Clymo and Hayward, 1982). The difference in growth response between the two species may be due to their difference in topographic positions, and water saturation level. The higher elevation of the peat ridges resulted in *S. fuscum* having less access to the nutrient- enriched water. Hummock species, such as *S. fuscum* are well adapted to dry conditions (Andrus, 1986). In period of drought and lower water table level, *S. fuscum* will use passive transport to conduct water though capillary uptake (Clymo and Hayward, 1982; Thompson and Waddington, 2008 Precipitation levels were high and relatively constant throughout the 2015 growing season (McCarter, 2016), therefore, *S. fuscum* may have retained water through retention of precipitation water rather than from increased pore-water pressure causing passive capillary uptake of the fertilized water. Therefore, the greater precipitation levels and the ridge's height above the fertilized water table, help explain why there was no difference in *S. fuscum* productivity rates between the experimental and reference fens in 2015 or 2016 (Table 2), and why the nutrient content (PC1 and C:N ratio) of surface level *S. fuscum* was also not significantly different between fens .

S. rubellum, in contrast, grows in dense carpets closer to the water table (Clymo and Hayward, 1982). Our results showed *S. rubellum* had significantly greater productivity within the first 50 m than the reference fen levels in 2015 and in 2016. Water table levels, and surface water discharge in the experimental fen were average throughout 2014, however, were very high throughout summer of 2015 (McCarter, 2016). In 2015, the first few ponds and ridges of the

experimental fen were experiencing high hydrological connectivity (McCarter, 2017). The pore water saturating the low-lying *S. rubellum* within the experimental fen would have contained more of the added nutrients, explaining the greater growth of *S. rubellum* strands within the first 50 m away from the point source of nutrient input. We suspect that the low-lying preferential flow paths connecting Pool 1, and Pool 2 allowed for transportation and distribution of the elevated nutrient water to reach all *S. rubellum* sample sites within the first 50 m of the experimental fen. *S. rubellum*, being a carpet-forming species, has high shoot and spreading branch density (Clymo and Hayward, 1982), and thus excellent water retention capabilities (Rydin and McDonald, 1985). High water retention capability would allow for continued uptake of nutrients into the new-grown tissues accounting for the continued high growth rates throughout the 2016 growing season and higher nutrient content.

We had also hypothesized that increasing nutrients within the experimental fen would increase the rate of *Sphagnum* decomposition, however, we did not find a significant difference in decay rates between the two sites. Our decomposition results did not support our hypothesis.

Many environmental factors influence *Sphagnum* decomposition rates, for example, temperature (Sjörs, 1959), microbial community (Thormann *et al*., 2004), degree of oxygenation (Johnson and Damman, 1993), litter chemistry, and nutrient availability (Aerts *et al*., 1992). These environmental factors also differ between hummock, carpet, and hollow habitats (Andrus *et al*., 1983). For example, hummock and hollow *Sphagnum* species differ in chemical composition where hummock species contain more complex recalcitrant organic matter (uronic acids and polyphenolic compounds) than hollow species (Clymo, 1963; Kälviäinen and Karunen, 1984; Johnson and Damman, 1991). Therefore, this variation in litter chemistry as well as the variation in moisture gradient between hummock and flat carpet *Sphagnum* habitats led us to

hypothesize that *S. rubellum* would have greater mass loss after the year incubation than *S. fuscum* in both fens. As nitrogen content within *Sphagnum* litter increases, the decay rate also increases (Clymo, 1965; Heal *et al*., 1978; Coulson and Butterfield, 1978). Therefore, we predicted that because the experimental fen was also receiving high nutrient loading, the increased N would only exaggerate this increase in mass loss to decomposition of *S. rubellum* litter.

We found that within both fens *S. fuscum* and *S. rubellum* decomposed at similar rates. Mean *S. rubellum* decomposition rate within the experimental fen (1.6 g dm^{-2} $year^{-1}$) was slightly greater than the reference fen mean (1 g dm^{-2} $year^{-1}$). Coulson and Butterfield (1978), found that increasing the nitrogen content within the living *Sphagnum* plants, increases the rate of decay when the plants die. But ultimately, Johnson and Damman (1993) found that the litter chemical composition is what limits decay regardless of the microhabitat (moisture or oxygenation level) that the litter is subjected to. Perhaps the *S. fuscum* and *S. rubellum* litter chemistry and nutrient content was not different enough from one and other to make a significant difference in the decay rates because the *Sphagnum* tissues collected for the decomposition received only one year of nutrient exposure prior to harvest, while the *Sphagnum* tissues harvested for nutrient analysis were exposed to two years of nutrient additions.

The pattern of nutrient content within the 2016 *Sphagnum* tissues support our nutrient content hypothesis. *S. fuscum* nutrient levels showed no difference between fens, *S. rubellum* showed border line significant difference between fens, and *S. majus* showed a strong significant increase in nutrient content within the experimental fen. This pattern indicates that nutrient uptake and retention was strongly linked to the ecological moisture gradient from hummock to hollow (less saturated to fully saturated). This result was expected because the nutrients were

added directly to the surface water table level, therefore, the *Sphagnum* species with the closest interaction with the water table would have the greatest ability to uptake those nutrients. This result may not have occurred if the nutrient additions were deposited aerially and evenly throughout the fen, as the case would be with peatland fertilization experiments that simulate atmospheric deposition of nutrients.

When assessing productivity rates across the hydrological gradient (distance from the point source), we can see that the zone most impacted by the added nutrients ends at about the 50 m mark. This distance covers from Pool 1 to the third ridge, which indicates that sample locations beyond the third ridge may not be receiving elevated levels of nutrients. Our nutrient data confirms that beyond the 70 - 100 m mark within the experimental fen, nutrient concentrations remain comparable to reference fen levels. These results parallel that of the hydrological transport of the wastewater nutrients within the pore water found by McCarter (2016). Our observed plant ecological response to the added wastewater nutrient can confirm that the pool-ridge-pool morphology of these ribbed fens appears to successfully polish and immobilize the added nutrients.

Throughout this experiment, *Sphagnum* productivity remained greater than the rate of *Sphagnum* decay. This result was exaggerated within the first 50 m downstream from the nutrient input, as the rate of productivity far exceeded the rate of decay. Therefore, an increase in nutrients leads to an increase in formation of peat, which translates to an increase in carbon storage. Increasing the capacity for these northern peatlands to store greenhouse gases would enhance an existing valuable ecosystem service. However, our results are an indication of the short-term ecological trends within these subarctic peatlands. Other peatland fertilization experiments, which maintained long-term nutrient additions have concluded that *Sphagnum*

decay rates increase with increasing nutrient content (Clymo, 1965; Heal *et al*., 1978), and in the long-term (> 5 years), *Sphagnum* decline in abundance, as they become out competed by other vascular plants, such as grasses and shrubs (Bubier *et al*., 2007; Bragazza *et al*., 2004; Berendse *et al*., 2001).

The key question remaining is how long can the northern peatlands of the HBL successfully uptake the added nutrients and remain *Sphagnum* dominant? Further research into the length of time these peatlands can sustain high nutrient loading before significant changes to the vegetation community would be highly beneficial. Additional monitoring of the productivity decomposition of the main peat forming vegetation would be useful, as would direct monitoring of the carbon flux within these peatlands if they are to be exposed to high nutrient loading long term. It is important for the HBL landscape to remain a globally significant carbon storage reservoir rather than switch to becoming a carbon source. The biological evidence provided by this study, as well as the hydrological evidence provided by McCarter (2016), suggests feasibility of these northern peatlands to polish treated domestic wastewater. Collaborators are conducting further research on the biogeochemical interactions resulting from adding wastewater into these peatlands, and will provide additional evidence towards assessing the environmental suitability of using the HBL peatlands to polish domestic wastewater.

Conclusion

Our findings suggest that *Sphagnum* within the HBL peatlands were nutrient deprived as providing them with additional nutrients over the course of two growing seasons allowed for increased productivity with no significant rise in decomposition. Variations in soil moisture level and microtopographic position relative to the water table plays a large role in accounting for the

differences in growth response and nutrient uptake between the *Sphagnum* species. Low-lying *Sphagnum* species, such as the dominant carpet and hollow species, were responsible for the uptake and immobilization of the nutrients.

Short-term high nutrient loading of the northern ribbed fen increased the formation of highly recalcitrant peat, indicating the potential for enhancement of the carbon sequestration ecosystem service, which the HBL peatlands are well known for providing. However, the long-term ecological balance between primary productivity and decomposition for this landscape remains speculative. Further research on the time line for maintaining a recalcitrant *Sphagnum* dominant peatland following press nutrient applications is required, along with investigation of possible biogeochemical interactions associated with the addition of nutrients.

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Literature Cited

Abraham, K.F., & Keddy, C.J. (2005). The Hudson Bay Lowland. *In* L.H. Fraser and P.A. Keddy (eds.). *The world's largest wetlands: Ecology and conservation*. Cambridge, UK: Cambridge University Press. pp 118-148.

Aerts, R., Wallén, B., Malmer, N., & De Caluwe, H. (2001). Nutritional constraints on Sphagnum-growth and potential decay in northern peatlands. *Journal of Ecology*, **89**(2), 292-299.

Aerts, R., Wallen B., & Malmer, N. (1992). Growth-limiting nutrients in Sphagnum dominated bogs subject to low and high atmospheric nitrogen supply. *Journal of Ecology* **80**, 131–140

Andrus, R., Wagner D.J., & Titus, J.E. (1983). Vertical distribution of Sphagnum mosses along hummock-hollow gradients. *Canadian Journal of Botany*, **61**, 3128-3139.

Andrus, R. E. (1986). Some aspects of Sphagnum ecology. *Canadian Journal of Botany*, **64**(2), 416-426.

Berendse, F., Van Breemen, N., Rydin, H., Buttler, A., Heijmans, M., Hoosbeek, M. R., ... & Wallén, B. (2001). Raised atmospheric CO2 levels and increased N deposition cause shifts in plant species composition and production in Sphagnum bogs. *Global Change Biology*, **7**(5), 591-598.

Bragazza, L., Tahvanainen, T., Kutnar, L., Rydin, H., Limpens, J., Hájek, M., ... & Iacumin, P. (2004). Nutritional constraints in ombrotrophic Sphagnum plants under increasing atmospheric nitrogen deposition in Europe. *New Phytologist*, **163**(3), 609-616.

Bran and Luebbe. (1986). Nitrate, Industrial Method 782–86T. Bran & Luebbe Analyzing Technologies, Elmsford.

Bubier, J. L., Moore, T. R., & Bledzki, L. A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, **13**(6), 1168-1186.

Clymo, R. S. (1963). Ion exchange in Sphagnum and its relation to bog ecology. *Annals of Botany*, **27**(2): 309-324.

Clymo, R. S. (1965). Experiments on breakdown of Sphagnum in two bogs. *The Journal of Ecology*, 747-758.

Clymo, R. S. & Hayward, P. M. (1982). The ecology of Sphagnum. *In Bryophyte ecology* (pp. 229-289). Springer Netherlands.

Clymo, R. S., & E. J. F. Reddaway. (1974). Growth rate of *Sphagnum rubellum* on Pennine blanket bog. *Journal of Ecology*, **62**(1), 191-196.

Clymo, R. S. (1984). The limits to peat bog growth. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **303**(1117), 605-654.

Coulson, J. C., & Butterfield, J. (1978). An investigation of the biotic factors determining the rates of plant decomposition on blanket bog. *Journal of Ecology*, **66**, 631-650.

Environment Canada. (2016). Canadian climate normal 1971-2000 station data. Government of Canada. Web. Retrieved on November 28, 2016. Available from http://climate.weather.gc.ca/climate_normals

Far North Science Advisory Panel. (2010). Science for a Changing Far North. The Report of the Far North Science Advisory Panel. A report submitted to the Ontario Ministry of Natural Resources.

Ferguson, P., Robinson, R.N., Press, M.C. & Lee, J.A. (1984). Element concentrations in five Sphagnum species in relation to atmospheric pollution. *Journal of Bryology*, **13**, 107-114.

Gorham, E. (1991). Northern peatlands: role in the carbon cycle and probable responses to climatic warming. *Ecological Applications*, **1**(2): 182-195.

Gorham, E., Janssens, J., & Glaser, P. (2003). Rates of peat accumulation during the postglacial period in 32 states from Alaska to Newfoundland, with special emphasis on northern Minnesota. *Canadian Journal of Botany*, **81**: 429-438.

Heal, O. W., Latter, P. M., & Howson, G. (1978). A study of the rates of decomposition of organic matter. In *Production ecology of British moors and montane grasslands* (pp. 136-159). Springer Berlin Heidelberg.

Johnson, L. C., & Damman, A. W. (1991). Species-controlled Sphagnum decay on a south Swedish raised bog. *Oikos*. **61**: 234-242.

Johnson, L. C., & Damman, A. W. (1993). Decay and its regulation in Sphagnum peatlands. *Advances in Bryology*, **5**, 249-296.

Kadlec, R. H. (2009). Wastewater treatment at the Houghton Lake wetland: Hydrology and water quality. *Ecological Engineering*, **35**, 1287–1311. doi:10.1016/j.ecoleng.2008.10.001

Kadlec, R. H., & F. B. Bevis. (2009). Wastewater treatment at the Houghton Lake wetland: Vegetation response. *Ecological Engineering*, **35**(9), 1312-1332.

Kadlec, R. H., & Wallace, S. D. (2009). Treatment Wetlands (2nd ed.). Boca Raton, FL: CRC Press, Taylor & Francis Group.

*Kälviäinen, E., & Karunen, P. (1984). On the growth, senescence, and decay-resistance of Sphagnum mosses. Proc. 7th Int. Peat Congr. Dublin. **4**: 263-275.*

Keddy, P. A., L. H. Fraser, A. I. Solomeshch, W. J. Junk, D. R. Campbell, M. T. K. Arroyo, & C. J. R. Alho. (2009). Wet and wonderful: the world's largest wetlands are conservation priorities, *BioScience*, **59**(1), 39-51.

Kimmel, K., & Mander, Ü. (2010). Ecosystem services of peatlands: Implications for restoration. *Progress in Physical Geography*, **34**(4), 491-514.

Larmola, T., Bubier, J. L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E., ... & Moore, T. R. (2013). Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global Change Biology*, **19**(12), 3729-3739.

Lee, P., M. Hanneman, J. Gysbers, R. Cheng, & W. Smith. (2010). Atlas of Canada's Intact Forest Landscapes, 74 pp., Global Forest Watch Canada, Edmonton, Alberta. http://www.globalforestwatch.ca/publications/20100913A

Limpens, J., Berendse, F., Blodau, C., Canadell, J. G., Freeman, C., Holden, J., ... & Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: from local processes to global implications–a synthesis. *Biogeosciences*, **5**(5), 1475-1491.

Martini, I.P. (2006). The cold climate peatlands of the Hudson Bay Lowland, Canada: Brief overview of recent work. Pp. 53-84 *in* Martini I.P., Cortizas, A.M., Chesworth, W. (eds.). Peatlands: Evolution and records of environmental and climate changes. Elsevier. Amsterdam, NL. 606 p.

McCarter, C.P.R. (2016). The hydrochemical fate and transport of treated domestic wastewater contaminants during a wastewater polishing experiment in a sub-arctic ladder fen peatland. Ph.D. thesis, Geography Department, University of Waterloo, Waterloo, Ontario, Canada.

McCarter, C. P., & Price, J. S. (2017). The transport dynamics of chloride and sodium in a ladder fen during a continuous wastewater polishing experiment. *Journal of Hydrology*. **549**, 558-570.

McCarter, C. P. R., Branfireun, B. A., & Price, J. S. (2017). Nutrient and mercury transport in a sub-arctic ladder fen peatland subjected to simulated wastewater discharges. *Science of The Total Environment*, ***609***, 1349-1360.

Potapov, P., Yaroshenko, A., Turubanova, S., Dubinin, M., Laestadius, L., Thies, C., ... & Karpachevskiy, M. (2008). Mapping the world's intact forest landscapes by remote sensing. *Ecology and Society*, **13**(2).

Press, M.C., Woodin, S.J. & Lee, J.A. (1986). The potential importance of an increased atmospheric nitrogen supply to the growth of ombrotrophic Sphagnum species. *New Phytologist*, **103**, 45-55.

Price, J. S., & Maloney, D. A. (1994). Hydrology of a patterned Bog-Fen Complex in Southeastern Labrador, Canada. *Nordic Hydrology*, **25**, 313-330.

Quinton, W. L., Hayashi, M., & Pietroniro, A. (2003). Connectivity and storage functions of channel fens and flat bogs in northern basins. *Hydrological Processes*, **17**, 3665-3684.

Quinton, W. L., & Roulet, N. T. (1998). Spring and summer runoff hydrology in a patterned wetland. *Arctic and Alpine Research*, **30**(3), 285-294. doi:10.2307/1551976

Riley, J.L. (2011). Wetlands of the Ontario Hudson Bay Lowland: An Ontario overview. Toronto, Ontario: Nature Conservancy of Canada.

Ronkanen, A.-K., & Klove, B. (2009). Long-term phosphorus and nitrogen removal processes and preferential flow paths in northern constructed peatlands. *Ecological Engineering*, **35**. doi:10.1016/j.ecoleng.2008.12.007

Rydin, H., & McDonald, A. J. S. (1985). Tolerance of Sphagnum to water level. *Journal of Bryology*, **13**(4), 571-578.

Sjörs, H. (1959). Bogs and fens in the Hudson Bay Lowlands. *Arctic*, **12**(1), 2-19.

Sjörs, H. (1963). *Bogs and fens on Attawapiskat River, northern Ontario*. Canada Department of Northern Affairs and National Resources.

Spence, C., & Woo, M.-k. (2003). Hydrology of subarctic Canadian Shield: Soil-filled valleys. *Journal of Hydrology*, **279**(1-4), 151-166. doi:10.1016/S002-1694(03)00175-6

Thompson, D. K., & Waddington, J. M. (2008). Sphagnum under pressure: towards an ecohydrological approach to examining Sphagnum productivity. *Ecohydrology*, **1**(4), 299-308.

Thormann, M. N., Bayley, S. E., & Currah, R. S. (2004). Microcosm tests of the effects of temperature and microbial species number on the decomposition of Carex aquatilis and Sphagnum fuscum litter from southern boreal peatlands. *Canadian Journal of Microbiology*, **50**(10), 793-802.

Turetsky, M. R., Crow, S. E., Evans, R. J., Vitt, D. H., & Wieder, R. K. (2008). Trade-offs in resource allocation among moss species control decomposition in boreal peatlands. *Journal of Ecology*, **96**(6), 1297-1305.

Van Wijk, M. T., Williams, M., Gough, L., Hobbie, S. E., & Shaver, G. R. (2003). Luxury consumption of soil nutrients: a possible competitive strategy in above-ground and below-

ground biomass allocation and root morphology for slow-growing arctic vegetation?. *Journal of Ecology*, **91**(4), 664-676.

Verhoeven, J.T.A. & Schmitz, M.B. (1991). Control of plant growth by nitrogen and phosphorus in mesotrophic fens. *Biogeochemistr*y, **12**, 135-148.

Woodin, S.J. & Lee, J.A. (1987). The fate of some components of acidic deposition in ombrotrophic mires. *Environmental Pollution*, **45**, 61-72.

Yu, Z., J. Loisel, D. P. Brosseau, D. W. Beilman, & S. J. Hunt. (2010). Global peatland dynamics since the Last Glacial Maximum. *Geophys. Res. Lett*., **37**, L13402, doi:10.1029/2010GL043584.

Table 1. Spearman's correlation coefficients among *Sphagnum* productivity, decomposition, first principal component (PC1) of the nutrient data and the C:N ratio within *Sphagnum* collected from the experimental fen. *S. fuscum* is shown in the upper triangular matrix (grey) and *S. rubellum* in the lower triangular matrix (white). Correlation coefficients in bold are significant at $P < 0.05$ based on a two-tailed test.

	Productivity 2015 6 weeks	Productivity 2016 6 weeks	Productivity 2016 12 weeks	Decomposition 1 year	PC1	C:N ratio
Productivity 2015: 6 weeks	-	**0.652**	**0.600**	0.018	**0.559**	**-0.692**
Productivity 2016: 6 weeks	**0.803**	-	**0.914**	0.304	0.094	-0.332
Productivity 2016: 12 weeks	**0.838**	**0.982**	-	0.305	0.096	-0.308
Decomposition: 1 year	0.369	0.072	0.140	-	-0.138	-0.076
PC1	**0.741**	0.359	0.289	0.034	-	**-0.791**
C:N ratio	-0.463	-0.253	-0.214	-0.056	**-0.853**	-

Table 2. Analyses of variance for productivity and decomposition separated by species, as a function of the fen site (experimental or reference), distance downgradient and their interaction. Results with type I error < 10% are in bold.

			Productivity 2015 (6-weeks)			Productivity 2016 (6-weeks)			Productivity 2016 (12-weeks)			Decomposition 2015-2016		
Species	Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
S. fuscum	Fen	1	0.02	0.1	0.787	0.17	0.1	0.791	0.01	0.0	0.973	0.16	0.8	0.387
	Distance	1	0.49	2.1	0.169	2.72	1.2	0.289	2.75	0.6	0.452	0.39	1.9	0.190
	Fen*Distance	1	0.10	0.4	0.530	0.13	0.1	0.812	1.18	0.3	0.620	0.01	0.0	0.829
	Error	16	0.24			2.27			4.63			0.21		
S. rubellum	Fen	1	18.92	7.9	**0.014**	41.41	6.4	**0.023**	71.22	7.0	**0.019**	1.35	3.5	**0.082**
	Distance	1	2.85	1.2	0.294	16.98	2.6	0.127	26.31	2.6	0.129	0.36	0.9	0.353
	Fen*Distance	1	8.57	3.6	**0.079**	14.60	2.2	0.155	27.36	2.7	0.122	0.55	1.4	0.254
	Error	15	2.39			6.50			10.21			0.39		

Table 3. Correlation (loadings) between the original variables and the first two principal components from the principal component analysis (PCA). Correlations in bold are significant at a 5% type I error rate.

	PC 1	PC 2
TN	**0.953**	-0.042
TS	**0.949**	0.034
P	**0.961**	0.107
K	**0.957**	0.005
Ca	-0.020	**-0.998**
Mg	**0.898**	-0.133

Table 4. Analyses of variance for the first principal component of plant nutrient content and for the C:N ratio, separated by species, as a function of fen site (experimental or reference), distance downgradient, and their interaction. Results with type I error < 10% are bolded.

			PC1			C : N		
Species	Source	df	MS	F	P	MS	F	P
S. fuscum	Distance	1	0.018	0.20	0.659	69.5	0.17	0.681
	Fen	1	0.010	0.11	0.741	74.4	0.19	0.670
	Fen*Distance	1	0.134	1.53	0.232	483.6	1.22	0.285
	Error	18	0.087			397.5		
S. rubellum	Distance	1	0.29	0.45	0.513	1145.4	2.17	0.160
	Fen	1	2.42	3.70	**0.072**	621.5	1.18	0.294
	Fen*Distance	1	1.29	1.96	0.180	186.7	0.35	0.560
	Error	16	0.65			528.2		
S. majus	Distance	1	10.7	4.74	**0.045**	664.3	7.76	**0.013**
	Fen	1	49.7	21.99	**0.0002**	2005.6	23.44	**0.0002**
	Fen*Distance	1	8.0	3.56	**0.077**	89.5	1.05	0.322
	Error	16	2.3			85.5		

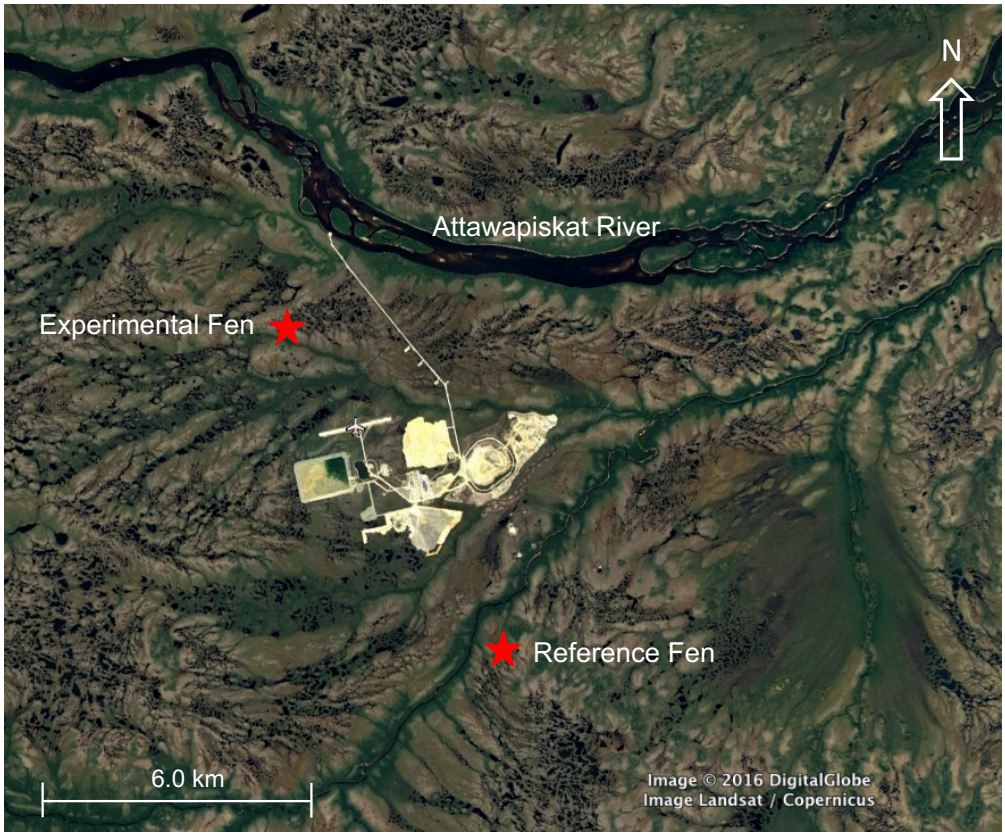


Figure 1. Google Earth satellite image of the De Beers Victor Mine site (June 2013), showing the experimental fen and the reference fen. The studied fens are 8.5 km apart.

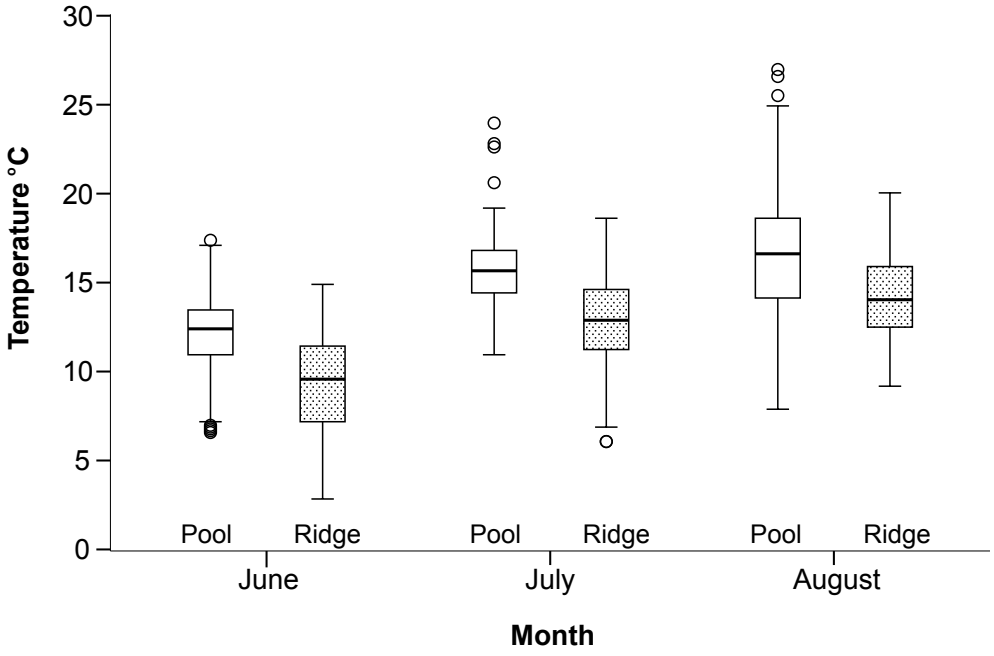
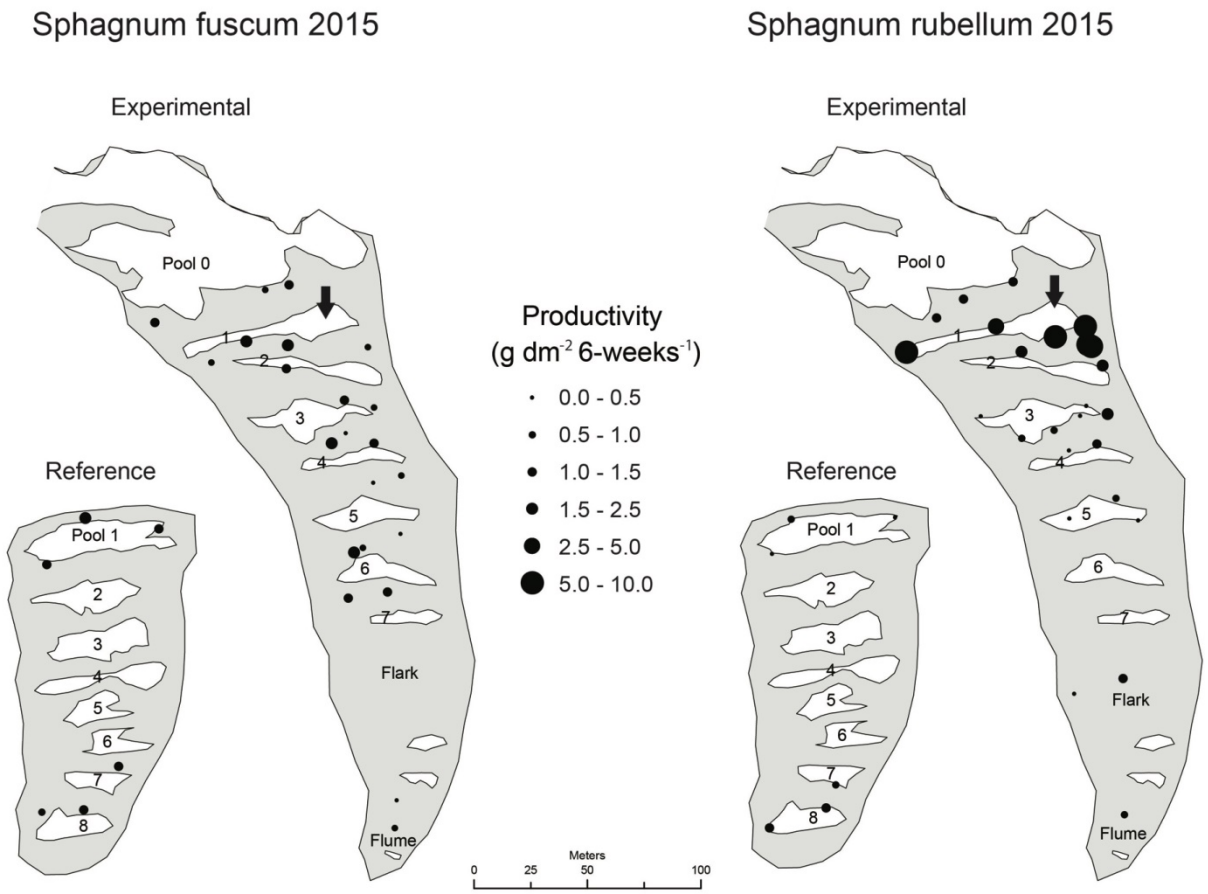
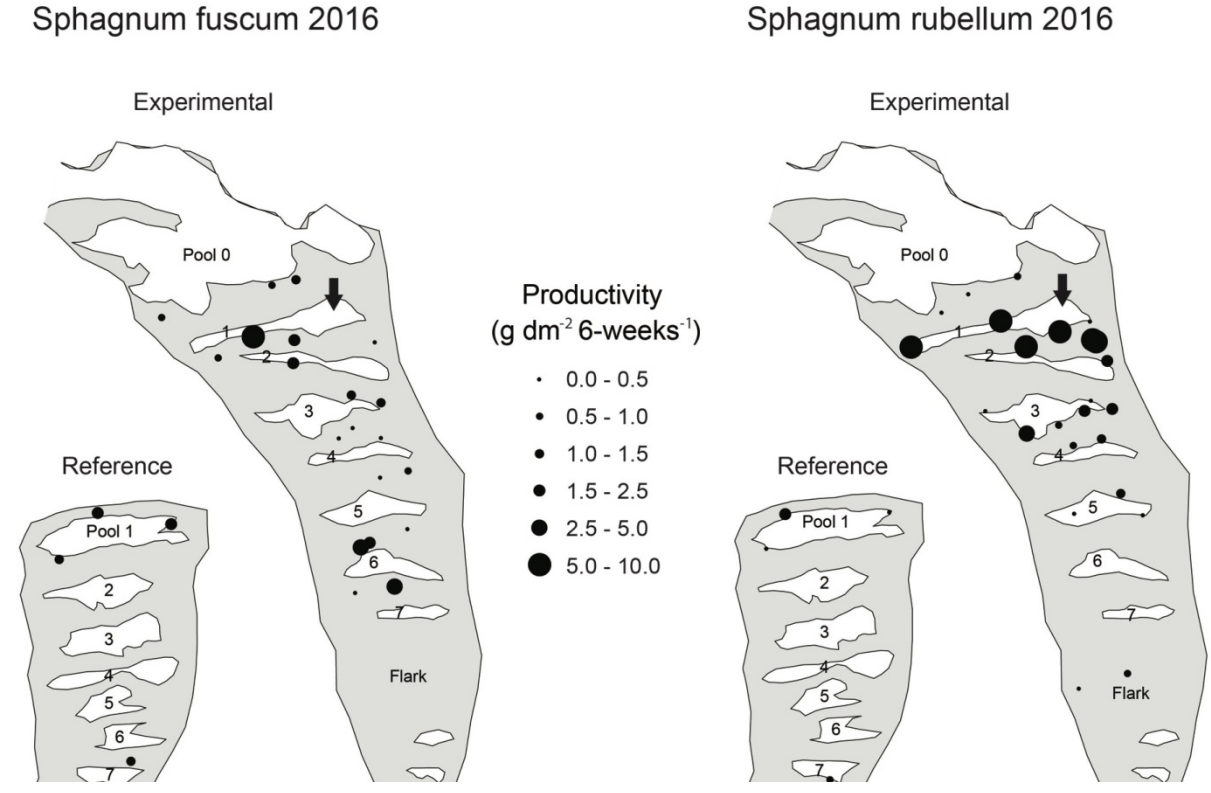


Figure 2. Box plots of monthly temperature of Pond 1(white) and Ridge 1 (grey) at 10 cm depth within the experimental fen during the 2016 growing season.


Sphagnum fuscum 2015
Sphagnum rubellum 2015
Experimental
Pool 0
1
2
3
4
5
6
7
Flark
Flume
Reference
Pool 1
2
3
4
5
6
7
8
Productivity
(g dm-2 6-weeks-1)
0.0 - 0.5
0.5 - 1.0
1.0 - 1.5
1.5 - 2.5
2.5 - 5.0
5.0 - 10.0
Meters
0
25
50
100


Sphagnum fuscum 2016
Sphagnum rubellum 2016
Experimental
Pool 0
1
2
3
4
5
6
7
Flark
Reference
Pool 1
2
3
4
5
6
7
Productivity
(g dm-2 6-weeks-1)
0.0 - 0.5
0.5 - 1.0
1.0 - 1.5
1.5 - 2.5
2.5 - 5.0
5.0 - 10.0

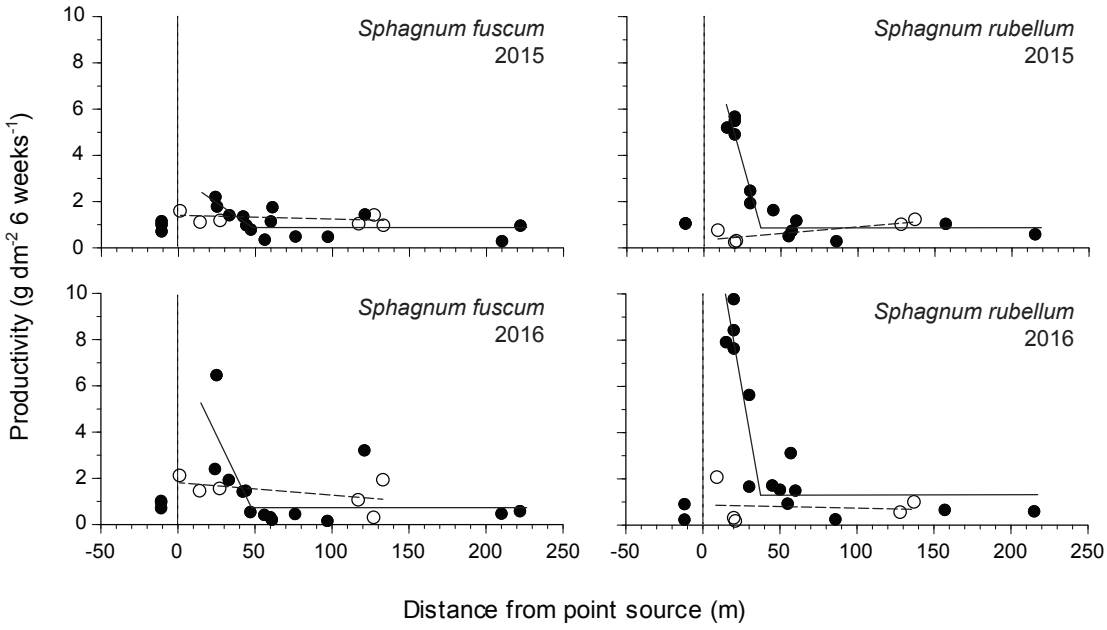
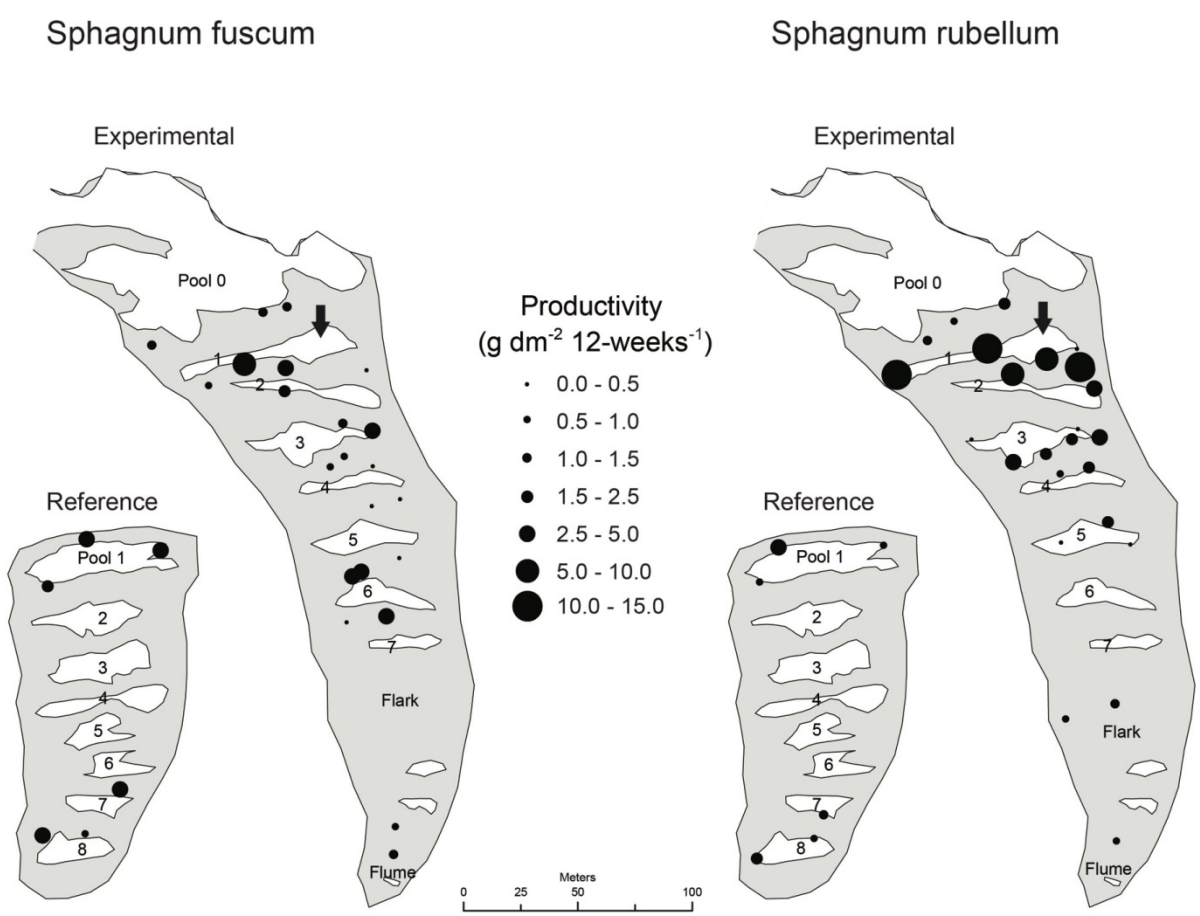
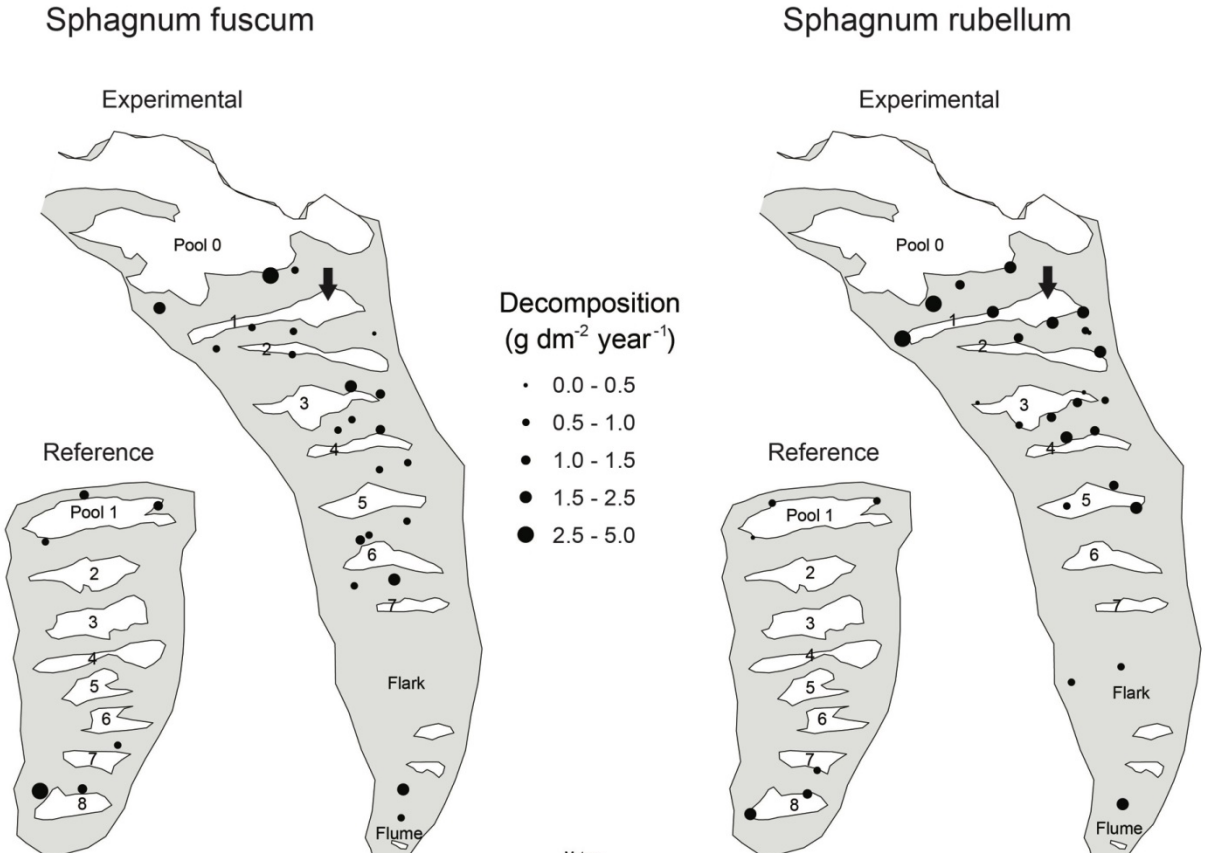


Figure 4. Productivity over 6 weeks during the growing season in 2015 (top) versus 2016 (bottom) for *Sphagnum fuscum* (left) and *Sphagnum rubellum* (right) as a function of distance from the discharge point in the experimental fen and the top edge of the fen in the reference fen. Solid lines and solid circles represent experimental fen data, and open circles and dashed lines represent the reference fen data. The vertical dotted line marks the point source input of nutrients in the experimental fen. Samples with negative distance values are located upgradient from the nutrient discharge point.


Sphagnum fuscum
Sphagnum rubellum
Experimental
Reference
Pool 0
Pool 1
Flark
Flume
Productivity
(g dm-2 12-weeks-1)
0.0 - 0.5
0.5 - 1.0
1.0 - 1.5
1.5 - 2.5
2.5 - 5.0
5.0 - 10.0
10.0 - 15.0
Meters
0
25
50
100


Sphagnum fuscum
Sphagnum rubellum
Experimental
Reference
Pool 0
Pool 1
Flark
Flume
Decomposition
(g dm-2 year-1)
0.0 - 0.5
0.5 - 1.0
1.0 - 1.5
1.5 - 2.5
2.5 - 5.0

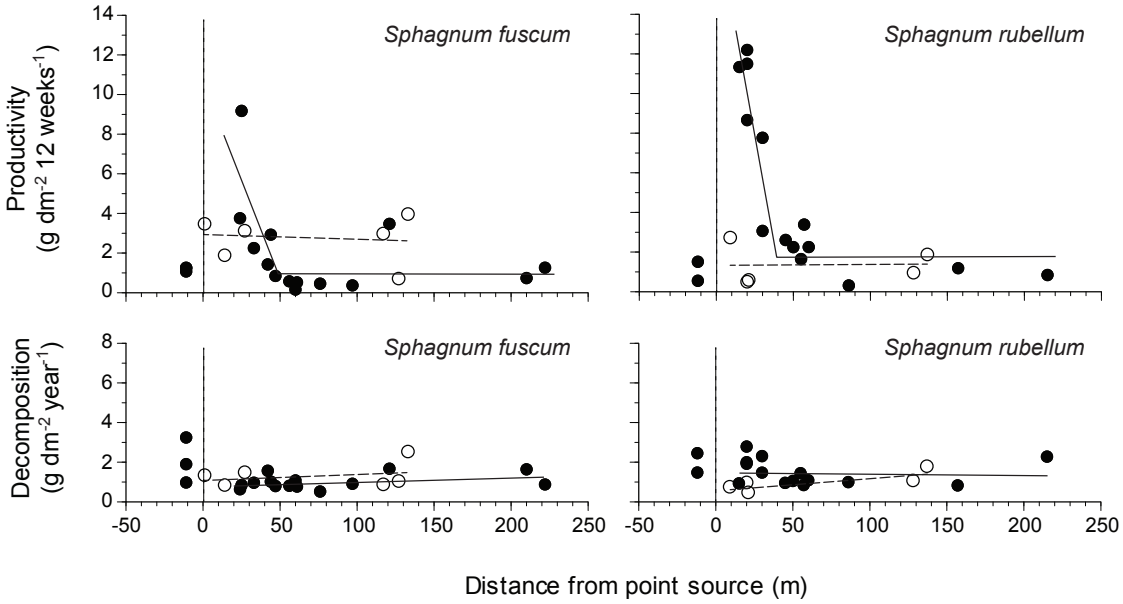


Figure 6. Linear regression plots of the productivity over 12 weeks during the growing season in 2016 (top) and annual decomposition (bottom) for *Sphagnum fuscum* (left) and *Sphagnum rubellum* (right) in the experimental fen (solid circles and lines) and the reference fen (open circles and dashed lines). The vertical dotted line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

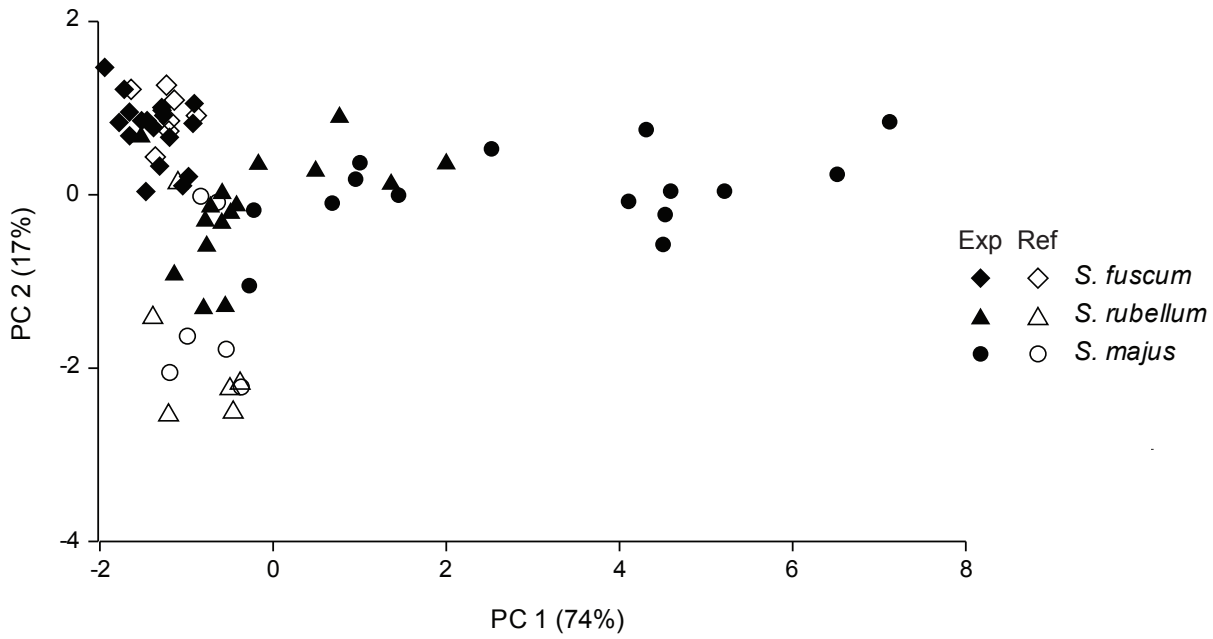


Figure 7. Principal component ordination plot of the nutrient content of all three *Sphagnum* species in the experimental fen (solid) and reference fen (open). The percent variation explained by principal component is shown in parentheses on each axis. Together they summarize 91% of the total variation of the nutrient content in the *Sphagnum* species.

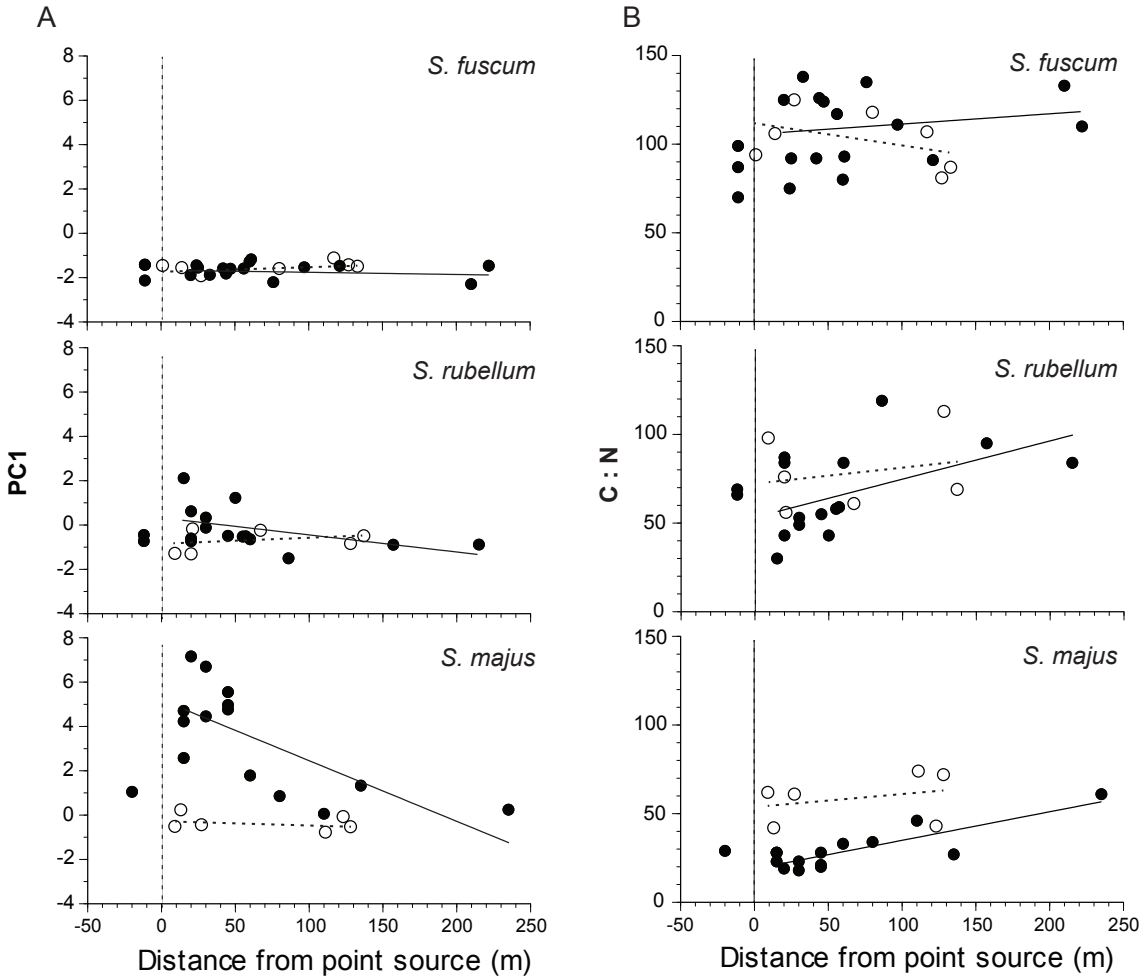


Figure 8. Linear regression plots of (A) the first principal component of *Sphagnum* nutrient content (PC1; left) and (B) the C:N ratio (right) in the experimental fen (solid circles and lines) and the reference fen (open circles and dashed lines). The vertical dotted line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

CHAPTER 2: Decomposition of *Sphagnum* peat from a ribbed fen receiving simulated treated wastewater: an incubation experiment along a nutrient loading gradient

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Abstract

Northern peatlands are dominated by *Sphagnum* moss. Slow decomposition in these peatlands leads to the sequestration and burial of carbon, providing a globally key ecosystem function. Resource extraction industries and small communities are growing in some northern peatland-dominated regions, so there is increasing interest in harnessing peatlands to polish wastewaters. In a previous collaborative research project, we set up a large-scale field experiment in the subarctic Hudson Bay Lowland to evaluate the effects of point-source additions of simulated treated wastewater into a ribbed fen, and we found a large productivity effect but no apparent impact on decomposition. We used a factorial peat incubation experiment to further investigate the decomposability of three dominant *Sphagnum* species representing hummock, carpet and hollow microsites. We tested whether these *Sphagnum* peats originating from the start versus the end of the experimental fen decomposed at different rates than in a reference fen. We also tested whether the nutrient dose, ranging from distilled water to up to ten times the dose in the field experiment, affected decomposition. We incubated samples under anaerobic conditions for 40 days, then aerobic conditions for 24 hours, then measured decomposability via microbial CO_2 and CH_4 production. Results show that hollow *Sphagnum* species consistently released more greenhouse gases than carpet or hummock species. Peat originating from the nutrient-enriched start of the experimental fen also had significantly greater emissions of CO_2 and CH_4. However, the addition of nutrients to the incubation jars did not significantly increase CO_2 or CH_4 production, and thus did not increase decay potentials. This suggests that *Sphagnum* peat from northern regions may be tolerant to high nutrient loadings associated with treated domestic wastewater and can retain their ability to resist decomposition, leading to continued carbon storage and net sequestration of greenhouse gasses.

Key words: Microbial respiration, methane production, carbon dioxide production, wastewater polishing, string fen, peatland, Hudson Bay Lowland, peatland microtopography

Introduction

Northern peatlands sequester carbon because their rate of primary productivity exceeds their rate of decomposition (Clymo and Hayward, 1982) and *Sphagnum* mosses are the key peat-forming species. Their slow decomposition leads to the sequestration and burial of carbon (Roulet, 2000) and provides a globally key ecosystem function. *Sphagnum* mosses occur across microtopographical gradients, from raised hummocks to flat carpets or lawns to depressed hollows or pools, in different species assemblages and under different decomposition environments (Andrus *et al*., 1983; Clymo and Hayward, 1982).

The decomposition of *Sphagnum* peat is first influenced by temperature (Sjörs, 1959; Thormann *et al*., 2004). Northern environments have discontinuous or continuous permafrost (Riley, 2011), leading to inherently slow rates of decay in northern peatlands (Bartsch and Moore, 1985; Johnson and Damman, 1993; Puranen *et al*., 1999). Warmer soil temperatures increase the rates of peat decay (Davidson *et al*., 2000; Ise *et al*., 2008). An incubation study using northern peat found that an increase in temperature from 12 °C to 22 °C caused up to a fourfold increase in CH_4, and a twofold increase in CO_2 production (Yavitt *et al.*, 1997). Peat warming and decomposition remains a main concern for climate change, although little consensus exists on the directional impacts on peat accumulation with climate warming (Davidson and Janssens, 2006).

Second, *Sphagnum* peat decomposition is heavily influenced by the water level and the consequent degree of aeration and redox potential (Yavitt *et al*., 1997). Peat decomposes two to three times faster under aerobic conditions compared to anaerobic conditions (Moore and Dalva, 1997; Bridgham and Richardson, 1992). The uppermost layer within a peatland is the acrotelm, which has fluctuating water table levels with at least periodic aerobic conditions, while the

underlying layer is the catotelm, which is fully saturated, with anaerobic conditions (Ivanov, 1981). Hummocks have a thick acrotelm (20-50 cm), while carpets are intermediate (5-20 cm) with intermittent water saturation, and hollows have little or no acrotolem (0-5 cm; Rydin and Jeglum, 2013). Peat is largely decomposed by aerobic or facultatively anaerobic bacteria and fungi, which metabolize the *Sphagnum* litter (Clymo and Hayward, 1982; Thormann *et al*., 2004), producing CO_2 or CH_4 as end products. CH_4 production (methanogenesis) occurs almost exclusively under low aerobic or anaerobic conditions, often just below the water table within the peat profile (Nillson and Bohlin, 1993; Bubier and Moore, 1994). Measuring the amount of microbial CO_2 and CH_4 production from peat has been widely used to quantify the rate of peat decomposition (Singh and Gupta, 1977).

Third, the chemistry of the *Sphagnum* litter plays a large role in its decomposition (Andrus, 1986; Bragazza *et al*., 2006). Hummock species contain a greater concentration of complex highly-recalcitrant uronic acids and polyphenolic compounds than hollow species (Clymo, 1963; Kälviäinen and Karunen, 1984; Johnson and Damman, 1991; Turetsky *et al*., 2008), so hummock species decompose more slowly (Clymo and Hayward, 1982; Andrus, 1986; Johnson and Damman, 1991; Hogg, 1993). Furthermore, younger *Sphagnum* litter near the surface decays more rapidly than deeper and older litter because the younger material has more easily degradable organic compounds, leaving the more complex and highly recalcitrant compounds in deeper peat layers (Clymo, 1984; Johnson and Damman, 1991; Hogg *et al*., 1992; Hogg, 1993). The degree of nutrient uptake within the new growth and fresher *Sphagnum* tissues has a strong impact on the decay potential of peat material as it becomes litter (Bartsch and Moore, 1985; Johnson and Damman, 1993; Hogg, 1993).

Finally, nutrients are in short supply in northern peatlands (Riley, 2011), so their addition, particularly of nitrogen, have been shown to intensify and increase the rates of *Sphagnum* decomposition (Berendse *et al.* 2001; Van Wijk *et al.* 2004). Laboratory incubations of peat have found that N and P additions increase the decay potential of *Sphagnum* peat (Hogg *et al*. 1994). In a four-year field experiment, however, Aerts *et al*., (2001) found that N or P additions had little effect on CO_2 production at high or low N sites. In another experiment, after five years of peatland fertilization, *Sphagnum* biomass and peat formation were significantly reduced (Bubier *et al*. 2007; Larmola *et al*. 2013). Ambiguities remain regarding the effects of nutrient additions on the rate of *Sphagnum* decay, and results differ depending on the concentration of nutrient amendment, temperature, oxygenation and water saturation level, differences in litter chemistry, and particularly, variations between *Sphagnum* species (Aerts *et al*., 1992; Bridgham and Richardson, 1992; Yavitt *et al*., 1997; Turetsky *et al*., 2008).

Northern peatlands provide other regulating ecosystem services of direct benefit to humans besides carbon sequestration and burial, including climate regulation, erosion protection, water quantity regulation and water filtration and purification (Kimmel and Mander 2010). We have commonly used the filtering function of marshes to polish treated domestic wastewaters (Kadlec and Wallace 2009), but we have less often used northern peatlands to polish wastewater (Kadlec 2009; Ronkanen and Klove 2009). Resource extraction industries and small communities are growing in some peatland-dominated regions (Far North Science Advisory Panel 2010), so there is increasing interest in harnessing peatlands to polish wastewaters.

Along with collaborative partners, we set up a large-scale field experiment in the subarctic Hudson Bay Lowland to evaluate the effects point source additions of simulated treated wastewater into a ribbed fen (McCarter, 2016). The fen effectively polished the nutrients over a

two-year period (McCarter, 2016). *Sphagnum* growth was strong in response, but we found no apparent impact of the increased nutrient loading on decomposition in the field (Chapter 1 in this thesis). Would the peat produced in this experimental fen decompose more or less rapidly if we changed the nutrient dose? If decomposition rates increased with higher nutrient amendments, this could have large consequences on the hydrology, structure, composition and water polishing function of treatment fens.

We used a laboratory incubation study to determine the influence of peat origin in the experimental fen versus subsequent nutrient loading on the decay potentials for three species of *Sphagnum* representing hummock, carpet and hollow environments. We hypothesized that a hummock-forming species (*Sphagnum fuscum*) would decay more slowly than a carpet species (*Sphagnum rubellum*), followed by a hollow species (*Sphagnum majus*). We then hypothesized that, following two seasons of high nutrient loading, peat originating from the start of the experimental fen would decay most rapidly, producing greater amounts of CO_2 and CH_4 than peat originating from the end of the experimental fen and a reference fen. Finally, we hypothesized that increasing nutrient amendments would further stimulate *Sphagnum* decomposition and CO_2 and CH_4 emissions, up to a certain threshold.

Methods

We collected the peat for this incubation experiment from an experimental ribbed fen receiving simulated secondarily-treated wastewater, near the De Beers Canada Victor Mine, located within the Attawapiskat River watershed of the Hudson Bay Lowland (HBL) in north-central subarctic Canada (52°49’08” N, 83°54’52” W; 80 m elevation). Details of the site and the experimental fen can be found in McCarter and Price (2017), and in Chapter 1 of this thesis. The simulated

wastewater was specially formulated to mimic the secondarily-treated wastewater effluent from the Victor Mine camp, and would also be typical of a small isolated community in northwestern Ontario. Briefly, the experimental fen received a continual input of simulated wastewater from a point source for 51 days in the summer 2014 and 41 days in summer 2015 at a rate of 38 m^3 day^{-1}. The field loading concentrations of nutrients were 27.2 mg L^{-1} of NO_3^-, 9.1 mg L^{-1} of NH_4^+, 7.4 mg L^{-1} of PO_4^{-3}, 24.5 mg L^{-1} of K^+ and 27.2 mg L^{-1} of SO_4^{-2}. A sodium chloride salt tracer was also added, so the experimental fen also received 25.3 mg L^{-1} of Na^+ and 47.2 mg $L^{-1}$of Cl^- (McCarter, 2016).

We collected surface peat (<~12 cm) on August 29 and 30, 2015 within three origin zones: the start of the experimental fen (Start EXP), the end of the experimental fen (End EXP), and from a nearby reference fen (REF). Surface water concentrations of NO_3^-, NH_4^+, PO_4^{-3}, and SO_4^{-2} remained elevated up to 50 m from the simulated wastewater point source (McCarter 2016), so we collected the Start EXP peat within the first 50 m downgradient from the point source, in and around the first two pool-ridge sequences. We collected End EXP peat from zones 140 to 250 m from the point source, because it had low nutrient concentration in surface waters, comparable to the reference fen. We collected REF peat samples close to the end of the reference fen. Within each zone, we collected peat from *Sphagnum fuscum* hummocks, *S. rubellum* carpets and *S. majus* pool habitats. We collected six samples per species per origin zone and bulked them together in plastic bags. We transported them cold to Laurentian University and kept them refrigerated for 5 weeks until we began the experiment. Prior to incubation experiment, we sorted through the material and removed and discarded any living *Sphagnum* sections of the strands to ensure that we only used dead (non-photosynthesizing) *Sphagnum* peat.

We set up a factorial experiment with the three species (*S. fuscum*, *S. rubellum*, and *S. majus*), three peat origins (Start EXP, End EXP, and REF) and five levels of nutrient amendments (0x, 0.5x, 1x, 2x, and 10x) of the loading levels in the field experiment. We used distilled water for the 0x control treatment and we diluted a concentrate of the same nutrient mix used in the field to obtain the other nutrient dosages. We conducted the incubations in 250 mL mason jars with tight sealing lids containing a rubber stopper in the centre to allow for gas extraction with a needle and syringe. We placed 10 g of fresh peat material into each jar, with 40 mL of the corresponding nutrient solution.

We first conducted a 40-day anaerobic incubation to simulate flooded conditions. We sealed the jars tightly to make the jar environment anoxic. We determined the remaining headspace volume of each individual jar, and then we flushed the headspace of each jar with N_2 gas using a Yellow Jacket® vacuum pump (SuperEvac 8) to ensure there was no other gases present prior to incubation. We placed the jars in a BioChambers® growth chamber (model AC-60), with no light and set to a constant 15 °C, which was the average air temperature during the 2015 growing season at De Beers Victor Mine. We extracted samples on days 1, 3, 7, 17 and 40, although we only present the day 40 results here. To extract the gas, we used 10 mL plastic syringes with needles to pierce through the rubber stopper in the jar lids. We first injected 10 ml of N_2 gas into a jar, flushed the syringe three times and then drew 10 ml of gas from the sample into the syringe. We ran the gas samples through a SRI model 8610C Gas Chromatograph® using PeakSimple® software within 48 hours after each sampling event. We also ran a standard with known CO_2 and CH_4 every 10 samples for quality control measures. Using the gas standards as a conversion factor and the known headspace volume within each jar, we used the ideal gas law equation to solve for the mass of CO_2 and CH_4 gas produced within each jar. We corrected our

data to account for the dilution factor generated by adding 10 mL of N_2 gas to the headspace of each jar at each sampling event.

On day 41, we conducted a 24-hour aerobic incubation using the same jars to mimic what would occur in the natural environment during a period with a lower water table. We opened the sealed jars and ran a high-powered fan over top for 10 minutes to flush the jar headspace with room air. After the aeration, we sealed the jars again and put them back in the growth chamber under dark conditions at 15 °C. This time when sampling the gas, we filled the syringes with room air prior to sampling, rather than N_2 gas. We sampled the headspace gas from each jar one hour after the aeration, and after 12 and 24 hours. We measured for CO_2 using the same instrument, method quality control procedure and calculations as above. After this incubation, we oven-dried the peat at 70 °C for 48 hours and weighed it. We then expressed the CO_2 and CH_4 mass on a dry mass basis.

We analyzed for responses in 40-day anaerobic CO_2 and CH_4 and 24-hour aerobic CO_2, using Statistica® version 10 and a 5% type I error rate. We log-transformed all the data. We first analyzed for species effects using one-way analyses of variance. We then conducted factorial analyses of variance separately by *Sphagnum* species to analyze for significant effects of peat origin and nutrient amendment and their interaction. We used post-hoc Tukey tests to further examine significant effects.

Results

Sphagnum species had significantly different CO_2 respiration after 40 days under anaerobic conditions, across all peat origins and nutrient amendments ($P < 0.0001$; Figure 1a). *S. fuscum* had the lowest anaerobic respiration of CO_2 (mean:7000 μg g dry mass^{-1}), and it differed

significantly from both *S. rubellum* and *S. majus* (both: Tukey $P < 0.0001$), but *S. rubellum* and *S. majus* were not significantly different from each other (Tukey $P = 0.276$; *S. rubellum* mean: 9930 μg g dry mass^{-1}; *S. majus* mean:11190 μg g dry mass^{-1}). The CO_2 respiration of *S. fuscum* peat after 40 days of anaerobic conditions strongly depended on the origin of the peat ($P = 0.0002$; Table 1); the peat from the start of the experimental fen had significantly greater respiration than the peat from both the end of the experimental fen and the reference fen, which were not different from each other (Figure 1a). However, nutrient amendment had no significant effect on the anaerobic microbial respiration of *S. fuscum* peat, nor was there any interaction with peat origin (Figure 2a). We found a similar pattern for *S. rubellum*. Its anaerobic respiration also depended on the origin of the peat ($P = 0.0002$), again with more CO_2 production for peat originating from the start of the experimental fen than from either the end of experimental fen or reference fen. Nutrient amendment again had no effect on the anaerobic CO_2 respiration of *S. rubellum* peat, again with no interaction. In contrast to the other species, the anaerobic CO_2 respiration of *S. majus* peat was not affected by its origin nor by any nutrient amendment.

When we examined the subsequent aerobic CO_2 respiration from the remnant peats over 24 hours, it was only half an order of magnitude less across all species than the anaerobic CO_2 respiration over 40 days (Figure 1b). All three *Sphagnum* species now strongly differed from each other (all species: Tukey $P < 0.0001$), with *S. fuscum* having the lowest 24-hour CO_2 aerobic respiration rate (mean: 1670 μg g^{-1} dry mass), followed by *S. rubellum* (mean: 2700 μg g^{-1} dry mass), and *S. majus* (mean: 3400 μg g^{-1} dry mass). The aerobic CO_2 respiration from *S. fuscum* peat did not depend on the origin of the peat (Table 1), but it did depend on the level of nutrient amendment ($P < 0.0001$; Figure 2b), and there was a significant peat origin by nutrient interaction. Peat originating from the start and end of the experimental fen with 10x field-loading

concentration of nutrients, produced a significantly lower amount of CO_2 that the 0x, 0.5x, 1x, or 2x field-loading nutrient concentrations, whereas the reference fen peat samples produced similar amounts of CO_2 across all nutrient amendment levels (Figure 3).

Aerobic CO_2 respiration of *S. rubellum* peat strongly differed depending on the peat origin ($P < 0.0001$; Table 1, Figure 1b); peat from the start of the experimental fen produced the greatest CO_2, while peat from the end of the experimental fen produced the least, and the reference fen produced intermediate levels. However, nutrient amendment had no significant effect on the aerobic CO_2 respiration of *S. rubellum* peat, nor was there any interaction with peat origin. *S. majus* behaved similarly. Aerobic CO_2 respiration of *S. majus* peat strongly depended on its origin ($P < 0.0001$; Table 1; Figure 1b), with peat from the start of the experimental fen producing the greatest CO_2, peat from end of the experimental fen producing the least, and the reference fen having intermediate levels (Figure 1b). Nutrient amendment had no significant nutrient effect on the aerobic CO_2 respiration of *S. majus* peat, nor was there any interaction with peat origin (Table 1).

When we examined the anaerobic CH_4 production after the 40-day incubation, it was between two and seven orders of magnitude lower than anaerobic CO_2 production (Figure 1c). All three species of *Sphagnum* were significantly different. *S. fuscum* produced extremely low amounts of CH_4 (mean: 0.21 $\mu g\ g^{-1}$ dry mass), while *S. rubellum* produced an intermediate amount (mean: 1.33 $\mu g\ g^{-1}$ dry mass), and *S. majus* produced the greatest CH_4 (mean: 21 $\mu g\ g^{-1}$ dry mass). The CH_4 production from *S. fuscum* peat did not depend on its origin, but it did depend on the nutrient amendment ($P = 0.010$; Table 1). CH_4 production was lowest under the 0x nutrient treatment, and increased to a peak at the 2x nutrient treatment, then CH_4 production declined when 10x nutrients were applied (Figure 2c). In contrast, the CH_4 production of *S.*

rubellum peat strongly depended on the origin of the peat ($P < 0.0001$); peat from the start of the experimental fen produced the most CH_4, while peat from the end of the experimental fen produced the least, and peat from the reference fen was intermediate (Figure 1c). Nutrient amendment also caused a difference in CH_4 production for *S. rubellum* peat ($P = 0.002$), but with a significant interaction with the peat origin ($P = 0.011$). CH_4 production dropped especially at the 10x nutrient amendment (Figure 2c), but this was especially evident for peat from the start of the experimental fen and did not occur at the end of the experimental fen (Figure 4). Finally, the CH_4 production from *S. majus* peat depended on the peat origin ($P = 0.0002$); the start of the experimental fen produced similar amounts of CH_4 as the reference fen, while the end of the experimental fen produced less CH_4 (Figure 1c). Nutrient amendment had no effect on CH_4 production from *S. majus* peat (Figure 2c) and no interaction occurred.

Discussion

We examined *Sphagnum* decomposability across three different *Sphagnum* species, collected from origins that differed in prior productivity, and along a gradient of nutrient amendments. We aimed to predict how *Sphagnum*-peat decomposition (mineralization of carbon) may change throughout the HBL peatlands should they become used to polish nutrients present within treated domestic wastewater.

We had first hypothesized that a hummock-forming species (*Sphagnum fuscum*) would decay more slowly than a carpet species (*Sphagnum rubellum*), followed by a hollow species (*Sphagnum majus*). Our results support our first hypothesis. *S. fuscum* produced the least CO_2 and CH_4, *S. rubellum* produced intermediate amounts, and *S. majus* produced the greatest, showing the most decay potential. Hummock forming species, such as *S. fuscum*, contain a

greater concentration by mass of highly recalcitrant complex organic compounds (polyphenolic compounds and uronic acids) than hollow species (Clymo 1963; *Kälviäinen* and Karunen 1984; Johnson and Damman 1991; Turetsky *et al*. 2008). In our results, the hollow>carpet>hummock decay pattern held true under anaerobic and aerobic conditions regardless of which location of peat origin or level of nutrient amendment. Our results support the conclusions from previous research that determined that variations in microbial decomposition across different *Sphagnum* species is primarily due to their variations in litter chemistry, specifically percentage of highly recalcitrant phenolic compounds (Berendse *et al*., 2001; Bragazza *et al*., 2005).

We also had hypothesized that, following two seasons of high nutrient loading, peat produced under higher nutrients, originating from the start of the experimental fen, would decay most rapidly, producing greater amounts of CO_2 and CH_4 than peat originating from the end of the experimental fen and a reference fen. Generally, we found that microbial CO_2 respiration and CH_4 production levels strongly differed by peat origin, supporting our second hypothesis. In most cases throughout this experiment, peat originating from the start of the experimental fen produced the most CO_2 and CH_4 outputs, showing greater decomposability compared to peat originating from the end of the experimental fen or reference fen. This result suggests that high productivity *Sphagnum* peat grown in locations exposed to a higher nutrient loading decompose more than low productivity *Sphagnum* peat, and supports the findings from other peatland fertilization studies (Aerts *et al*., 2001; Bubier *et al*., 2007). Increasing the N content within *Sphagnum* peat and lowering the C:N ratio leads to faster rates of decomposition (Coulson and Butterfield, 1978; Clymo and Hayward, 1982). Nutrient content results from our previous study (Chapter 1) confirm that all three species had significantly lower C:N values in the peat samples collected from the start of the experimental fen location. *S. fuscum* had 1.5x lower C:N values, *S.*

rubellum 3x lower, and *S. majus* samples had 3.5x lower C:N values at the start of the experimental fen compared to the end of experimental fen or reference fen levels (Chapter 1), showing interspecific variation in nutrient retention across these origins.

However, some results from this study were exceptions to the general trend with peat origin, specifically, *S. majus* CO_2 production under anaerobic incubation, and *S. fuscum* aerobic CO_2 and anaerobic CH_4 production, which showed no significant difference across peat origin (Table 1; Figure 1). For these exceptions, we can suggest that the intraspecific litter nutrient content within the peat from all three origins may not have been significantly different from one another. In the case of *S. majus* CO_2 production under anaerobic conditions, we suspect that the *S. majus* surface peat tissues collected from the start of the experimental fen were primarily composed of newer growth (younger) tissues that grew quickly, and may not have had time to accumulate (absorb) the field-loaded nutrients and therefore, N, P, K, and S concentration within those samples may have been similar to those from the non-fertilized older *S. majus* tissues found at the end of the experimental fen and reference fen.

Similarly, for *S. fuscum* aerobic CO_2 and anaerobic CH_4 production, we believe that while *S. fuscum* was in the field during the field-loading of treated wastewater nutrients experiment (2015 growing season), the fertilized water table stayed between 10 – 40 cm below the top surface of the high hummocks on the peat ridges where *S. fuscum* grows (McCarter, 2016), therefore, the start of the experimental fen *S. fuscum* tissues could have had comparable nutrient concentrations to the end of the experimental fen and reference fen tissues.

Interestingly, our experimental results did not support our third hypothesis proposing that with greater nutrient amendment levels would yield greater CO_2 and CH_4. We found that under anaerobic and aerobic incubation, the addition of nutrients to the incubation jars did not

significantly increase CO_2 or CH_4 production, and thus did not increase decay potentials. Similar studies within the literature contain mixed results regarding the effects of nutrient additions on peat decay. Some peat incubation studies found that treatments with N, and N and P, lead to significantly greater decomposition (Coulson and Butterfield 1978; Hogg *et al*. 1994; Aerts and Toet 1997). However, many other studies had results similar to this study where nutrient additions had no significant increase to microbial CO_2 and CH_4 emissions (Bartsch and Moore 1985; Williams and Silcock 1997; Hoosbeek *et al*. 2002). Amador and Jones (1993) and Aerts *et al*. (2001) found no increase in peat CO_2 respiration with increased P fertilization, however, in more nutrient poor ombrotrophic peatlands, both N and P are predicted to be limiting decay and P additions significantly increase CO_2 production (Aerts *et al*. 1992; Hogg *et al*. 1994).

Many past peatland fertilization experiments have nutrient fertilization rates at much lower doses than this study, making comparison of our results to theirs difficult. For example, the ombrotrophic peatland outside Ottawa Ontario (Mer Bleau bog) received N loading treatment ranging from 1.6 – 6.4 g N m^{-2} $year^{-1}$ (Bubier *et al*., 2007), and our field-loading N application level (1x nutrient amendment treatment) would be approximately 9.4 – 15.3 g N m^{-2} $year^{-1}$, and our 10x nutrient loading amendment level would equate to 94 – 153 g N m^{-2} $year^{-1}$.

Limpens and Berendse (2003), suggest that within some of the peatland fertilization studies where results showed no increase to decay, that the cause could be that the C:N ratio of the *Sphagnum* litter may not have decreased enough to see a positive effect on decay potentials. If this theory applied here to our study, we would suspect that *Sphagnum* tissues from the start of the experimental fen with 10x nutrient loading would have low C:N ratios and increase their decay potentials, yet we did not see this result. In fact, we noted a significant decrease in CO_2 emission from the *S. fuscum*, and a decrease in CH_4 emissions from *S. rubellum* at the highest

(10x) nutrient loaded level (Figure 2). This result implies a theoretical threshold where above that level of nutrient loading, microbial decay declines or is inhibited, and furthermore, these thresholds may differ depending on the *Sphagnum* species.

The theory of optimal ranges of nutrient ratios, (C:N, C:P, C:K, C:S), or critical ratios within *Sphagnum* peat has been mentioned as explanation to the variability of results within the literature regarding nutrient fertilization effects on *Sphagnum* decomposition (Moore *et al*., 2011; Wang *et al*., 2014). Lamers *et al*. (2000) and Berendse *et al*. (2001) suggest that low levels of N deposition, *Sphagnum* quickly absorb the added N, but under increasing input *Sphagnum* become N-saturated to the point where they lose their N filtering capacity. Bragazza *et al*., (2005) added to this theory by conforming that within regions with higher N deposition, the decreasing retention of N within the *Sphagnum* was accompanied by an exponential increase in the concentration of inorganic N in the surrounding pore water.

Perhaps a similar principle may apply for other nutrient ratios such as the C:P, C:K, and C:S ratios within peat where there is an optimal range where microbial metabolization occurs in greater amounts, and additions above that range may be toxic to microbes and could cause a decrease in respiration, thus decreasing their decay potential (Wang *et al*., 2014). Similarly, the 10x nutrient loading amendment may have contained enough ammonia (NH_4) to be toxic for the microbes, causing an inhibitory effect to microbial respiration. Waste water contains high concentrations of NH_4, and high concentrations have been determined to decrease microbial activity (Lee *et al*., 2000). Anthonisen *et al*., (1976) found that 10 – 150 mg L^{-1} of NH_4 was inhibitory to many heterotrophic microbes. The 10x nutrient amendment within this experiment contained 91 mg L^{-1} of NH_4, which falls within the identified rage of NH_4 toxicity, and therefore,

could be the cause for significantly less microbial respiration from the highest nutrient amendment level.

We know that *Sphagnum* species can vary in litter chemistry, specifically their carbon: polyphenol compound ratios (Kälviäinen and Karunen 1984; Turetsky *et al*. 2008). Bragazza *et al*. (2006), found that the C:N ratio within *Sphagnum* is strongly positively correlated with polyphenol concentrations, and the C:N ratio also positively correlates with other nutrient ratios such as C:P and C:K, and therefore, the C:N ratio is an excellent predictor of *Sphagnum* decomposability, and supports that variations to litter chemistry and nutrient content translates to changes in decay potentials.

In this study, *S. fuscum* decay was significantly lower at 10x nutrient loading levels. We suspect that the microbes may have become substrate limited as the amount of easily labile organic carbon may have been previously depleted. The remaining organic carbon was likely bound within highly recalcitrant polyphenolic compounds and unavailable to most microbes for easy mineralization. *S. fuscum* is a slow growing species, thus the ~10 cm of surface peat collected for this study would have been older in age and had a greater concentration of polyphenols relative to the faster growing (newer growth) of the *S. rubellum* and *S. majus* samples, particularly within the start of experimental fen location where growth rates were significantly faster than the end of experimental fen or reference fen (Chapter 2). Therefore, the differences in decomposition could be due to differences in chemical aging of the peat substrate, which was also the conclusion from a similar *Sphagnum* peat decomposition study (Belyea, 1996).

However, the pattern of increased nutrients causing decreased microbial respiration was location specific as it only occurred within the two experimental fen locations, and not within the

reference fen. Reference fen *S. fuscum* peat produced similar CO_2 outputs across all nutrient treatment levels (Figure 3). This result lead us to suspect that perhaps there were abiotic environmental factors (temperature, pH, or water level) that differed between the two ribbed fens sites allowing for some variations to either litter structure or litter chemistry, and allowed for more tolerance to higher levels of nutrient loading.

An environmental factor within the incubation environment that may have been altered with high nutrient loading is pH. Perhaps the 10x nutrient loading level caused a decrease to pH to the point where microbial respiration and *Sphagnum* decay was inhibited. Greater additions of NO_3, NH_4, PO_4, and SO_4 nutrients can increase soil acidity (Parchomchuk *et al.*, 1993). Preston *et al*., (2012) concluded that pH was a strong predictor of microbial activity within peatland soils. Other studies have concluded that microbial diversity and activity is greater at more neutral pH levels (6-7) rather than acidic pH levels (3-5) (Fierer and Jackson, 2006; Rousk *et al*., 2010). A limitation of our study was that we did not measure the pH of the pore water within the incubation jars throughout our experiment, therefore, we are unable to determine if there was a significant pH decrease within the 10x nutrient loading treatments, that may be contributing to the decrease in CO_2 outputs from *S. fuscum* and *S. majus* at the 10x nutrient amendment level.

Upon assessing CH_4 production across our nutrient amendment rates, we found that *S. fuscum* and *S. rubellum* produced the least amount of CH_4 when fertilized at the highest treatment level (10x) (Figure 2). This result also leads us to suspect that high nutrient loading may inhibit some pathways within the process of *Sphagnum* decay, and that the amount of nutrient amendment required to see this inhibition, differs between *Sphagnum* species, as *S. majus* produced a similar amount of CH_4 across all amendment levels. In this case, we predict that the high level of sulfate (SO_4^{-2}) within our simulated wastewater fertilizer is likely the cause

for the decrease in CH_4 production for *S. fuscum* and *S. rubellum*. S has been identified as playing an important role in the oxidation-reduction pathway within peatlands (Gauci *et al*. 2002; Gauci *et al*., 2004), and as a result, additions of SO_4^{-2} have been linked to a reduction or inhibition of CH_4 emissions (Kristjansson *et al*., 1982; Yavitt *et al*., 1987; Blodau and Moore, 2003). Briefly, under anaerobic conditions, when SO_4^{-2} is increased in availability, the dominant terminal respiration process will become more favorable for sulfate reducing bacteria rather than methanogens (methane producing bacteria) because SO_4^{-3} reduction requires less energy (Kristjansson *et al*., 1982; Yavitt *et al*., 1987). We presume that *S. majus* could sustain a consistent output of CH_4 across all nutrient amendment levels because it develops under anaerobic water saturated conditions (Clymo and Hayward 1982), which are also the conditions required for methanogens (Dunnfield *et al*., 1993) and thus as a species may have developed more favorable litter structure and chemical composition for methanogens in comparison to *S. fuscum and S. rubellum*. Furthermore, microbial CH_4 production is suspected to be more sensitive to environmental changes, for example temperature, pH, water level, and redox-oxidation potential than CO_2 production (Moore and Dalva, 1993; Lai, 2009). While the peat was within its incubation environment in the lab, we could control temperature and water level, however, prior to peat harvest while the moss was developing and under earlier stages of decomposition, these environmental factors were highly variable within the natural field conditions, which may account for variability of litter structure within a species or across peat origin.

Overall, *Sphagnum* decay potentials significantly differed across hummock, carpet, and hollow species, and across peat origins, however, we suspect that the key factor that varied between these three species and these three peat origins is their variations in litter chemistry. In

addition, because peat origin within this experiment was highly significant, we believe that nutrient uptake within the *Sphagnum* tissues while they are still active (photosynthetic) has a greater effect on the decay potential of the *Sphagnum* once it becomes litter than does adding additional nutrient amendments to the incubation environment once the *Sphagnum*-peat is already inactive (non-photosynthetic). Therefore, long-term in-situ (field-based) experiments studying the pattern of CO_2 and CH_4 emission from these three species in their respective microhabitats would be valuable to better understand their evolution of decay within their natural environments from the point of productivity to the point they begin to senesce.

Understanding how nutrient additions can change the decomposability of *Sphagnum* peat is critical for predicting further changes to the physiological structure of the peat within these HBL ribbed fens. Ribbed fens are desirable systems for wastewater polishing because of their pattern of raised peat ridges (hummocks) interspersed with hollow pools where the ridges impede hydrological flow and the nutrient deprived *Sphagnum* scavenge the added nutrients immobilizing it from the pore water further downstream (McCarter, 2016). Increases or decreases to the rate of decay within these peat ridges could change the hydrological properties of these mires, possibly rendering them less effective at polishing wastewater. Additional long-term research on how high hydrological loading and nutrient loading would change the physiological structure of these ribbed fens would be beneficial for providing further comment on the feasibility of these peatlands to continue to polish wastewater long term.

The number one ecosystem service provided by these northern HBL peatlands is their ability to store carbon because their net uptake of atmospheric CO_2 far exceeds their release of CO_2 or CH_4 back into the atmosphere. For these systems to remain a globally significant carbon sink, their rate of primary productivity must remain greater than their rate of decay. Our short-

term field study (Chapter 1) concluded that with point source nutrient application, productivity remained greater than decomposition. Within this short-term incubation study, higher nutrient loading levels did not significantly increase the rate of CO_2 or CH_4 emissions, which again suggests that these *Sphagnum* species are tolerant of high nutrient loadings.

Other long-term peatland fertilization experiments concluded that *Sphagnum* decay rates increase with increasing nutrient content (Clymo 1965; Heal *et al*., 1978), and *Sphagnum* decline in abundance as they become out competed by other vascular plants, such as grasses and shrubs (Bubier *et al*., 2007; Bragazza *et al*., 2004; Berendse *et al*., 2001). As grasses, sedges, and shrubs have faster rates of decomposition relative to *Sphagnum* (Rydin and Jeglum 2013), if these plant community shifts were to occur within the HBL peatlands, than long-term net carbon storage of these systems may diminished. Further field research on plant community shifts within the HBL peatlands experiencing higher nutrient loading would be beneficial.

Conclusion

The HBL peatlands are under increased pressure for mining development and anthropogenic influences on the landscape. Should the population of the far north Ontario increase, the need for wastewater polishing will also increase. Few studies have been conducted on the effects of adding point source nutrients that contain nutrients similar to what would be present within treated wastewater directly to a ribbed fen within a northern subarctic climate. We found that peat nutrient content (concentration of organic bound C, N, and P) within peat plays a large role in the decay potential for *Sphagnum*-based peat, and the optimal nutrient ratios within the litter likely differs at the species level. Highly recalcitrant hummock forming *Sphagnum* species are more resistant to decomposition than carpet and hollow species, however hummock species may

be more likely to leech labile inorganic nutrients while under high nutrient loading. We provide evidence that litter chemistry and nutrient uptake (and retention) within living *Sphagnum* has a greater influence on the decomposability of that material than fertilization to non-living *Sphagnum* peat. Our results support findings that the litter chemistry variables such as polyphenols: nutrient ratios, and C: nutrient ratios appear to be the primary variables controlling microbial decomposition of *Sphagnum* moss (Limpens and Berendse, 2003; Bragazza *et al*., 2006), and carbon mineralization rates (Yavitt *et al*., 1987).

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Literature Cited

Aerts, R., Wallen, B., & Malmer, N. (1992). Growth-limiting nutrients in Sphagnum-dominated bogs subject to low and high atmospheric nitrogen supply. *Journal of Ecology*, 131-140.

Aerts, R., & Toet, S. (1997). Nutritional controls on carbon dioxide and methane emission from Carex-dominated peat soils. *Soil Biology and Biochemistry*, **29**(11), 1683-1690.

Aerts, R., Wallén, B., Malmer, N., & De Caluwe, H. (2001). Nutritional constraints on Sphagnum-growth and potential decay in northern peatlands. *Journal of Ecology*, **89**(2), 292-299.

Amador, J., & Jones, R. D. (1993). Nutrient limitations on microbial respiration in peat soils with different total phosphorus content. *Soil Biology and Biochemistry*, **25**(6), 793-801.

Andrus, R., Wagner D.J., & Titus, J.E. (1983). Vertical distribution of Sphagnum mosses along hummock-hollow gradients. *Canadian Journal of Botany*, **61**, 3128-3139.

Andrus, R. E. (1986). Some aspects of Sphagnum ecology. *Canadian Journal of Botany*, **64**(2): 416-426.

Anthonisen, A. C., Loehr, R. C., Prakasam, T. B. S., & Srinath, E. G. (1976). Inhibition of nitrification by ammonia and nitrous acid. *Journal (Water Pollution Control Federation)*, **48** (5), 835-852.

Bartsch, I., & Moore, T. R. (1985). A preliminary investigation of primary production and decomposition in four peatlands near Schefferville, Québec. *Canadian Journal of Botany*, **63**(7), 1241-1248.

Belyea, L. R. (1996). Separating the effects of litter quality and microenvironment on decomposition rates in a patterned peatland. *Oikos*, **77** (3), 529-539.

Bridgham, S. D., & Richardson, C. J. (1992). Mechanisms controlling soil respiration (CO2 and CH4) in southern peatlands. *Soil Biology and Biochemistry*, **24**(11), 1089-1099.

Berendse, F., Van Breemen, N., Rydin, H., Buttler, A., Heijmans, M., Hoosbeek, M. R., ... and Wallén, B. (2001). Raised atmospheric CO2 levels and increased N deposition cause shifts in plant species composition and production in Sphagnum bogs. *Global Change Biology*, **7**(5): 591-598.

Blodau C, & Moore TR. (2003). Micro-scale CO2 and CH4 dynamics in a peat soil during a water table fluctuation and sulfate pulse. *Soil Biology and Biochemistry*, **35**, 535–547.

Bragazza, L., Tahvanainen, T., Kutnar, L., Rydin, H., Limpens, J., Hájek, M., ... & Iacumin, P. (2004). Nutritional constraints in ombrotrophic Sphagnum plants under increasing atmospheric nitrogen deposition in Europe. *New Phytologist*, **163**(3), 609-616.

Bragazza, L., Limpens, J., Gerdol, R., Grosvernier, P., Hajek, M., Hájek, T., ... & Rydin, H. (2005). Nitrogen concentration and δ15N signature of ombrotrophic Sphagnum mosses at different N deposition levels in Europe. *Global Change Biology*, **11**(1), 106-114.

Bragazza, L., Siffi, C., Iacumin, P., & Gerdol, R. (2006). Mass loss and nutrient release during litter decay in peatland: the role of microbial adaptability to litter chemistry. *Soil Biology and Biochemistry*, **39**(1), 257-267.

Bubier J. L. & Moore T. R. (1994). An ecological perspective on methane emissions from northern wetlands. *Trends in Ecology and Evolution*, **9**, 460-464.

Bubier, J. L., Moore, T. R., & Bledzki, L. A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, **13**(6), 1168-1186.

Clymo, R. S. (1963). Ion exchange in Sphagnum and its relation to bog ecology. *Annals of Botany*, **27**(2): 309-324.

Clymo, R. S. (1965). Experiments on breakdown of Sphagnum in two bogs. *The Journal of Ecology*, 747-758.

Clymo, R. S. & Hayward, P. M. (1982). The ecology of Sphagnum. *Bryophyte ecology* (pp. 229-289). Springer Netherlands.

Clymo, R. S. (1984). Limits to peat bog growth. *Phil. Trans. R. Soc*. London B 303: 605-654.

Coulson, J.C. & Butterfield, J. (1978). An investigation of the biotic factors determining the rates of plant decomposition on blanket bog. *J. Ecol*. **66**: 631-650

Davidson, E. A., Trumbore, S. E., & Amundson, R. (2000). Biogeochemistry: soil warming and organic carbon content. *Nature*, **408**(6814), 789-790.

Davidson, E. A., and Janssens, I. A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**(7081), 165-173.

Dunfield, P., Knowles, R., Dumont, R., & Moore, T. R. (1993). Methane production and consumption in temperate and subarctic peat soils: response to temperature and pH. *Soil Biology and Biochemistry*, **25**(3), 321-326.

Far North Science Advisory Panel. (2010). Science for a Changing Far North. The Report of the Far North Science Advisory Panel. A report submitted to the Ontario Ministry of Natural Resources.

Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America*, **103**(3), 626-631.

Gauci V, Dise N, & Fowler D. (2002). Controls on suppression of methane flux from a peat bog subjected to simulated acid rain sulfate deposition. *Global Biogeochemical Cycles*, **16**, 1–12.

Gauci V, Matthews E, Dise N *et al*. (2004). Sulfur pollution suppression of the wetland methane source in the 20th and 21st centuries. *Proceedings of the National Academy of Sciences USA*, **101**, 12583–12587; doi 10.1073/pnas.0404412101.

Heal, O.W., Latter, P.M. & Howson, G. (1978). A study of the rates of decomposition of organic matter. In: Heal, O.W. and Perkins,D .F. (eds), Production ecology of British moors and montane grasslands, Ecological Studies 27. Springer, NY, 136-186.

Hogg, E. H., Lieffers, V. J., & Wein, R. W. (1992). Potential carbon losses from peat profiles: effects of temperature, drought cycles, and fire. *Ecological Applications*, **2**(3), 298-306.

Hogg, E. H. (1993). Decay potential of hummock and hollow Sphagnum peats at different depths in a Swedish raised bog. *Oikos*, 269-278.

Hogg, E. H., Maimer, N., & Wallen, B. (1994). Microsite and regional variation in the potential decay rate of Sphagnum magellanicum in south Swedish raised bogs. *Ecography*, **17**(1), 50-59.

Hoosbeek, M. R., Van Breemen, N., Vasander, H., Buttler, A., & Berendse, F. (2002). Potassium limits potential growth of bog vegetation under elevated atmospheric CO2 and N deposition. *Global Change Biology*, **8**(11), 1130-1138.

Ise, T., Dunn, A. L., Wofsy, S. C., & Moorcroft, P. R. (2008). High sensitivity of peat decomposition to climate change through water-table feedback. *Nature Geoscience*, **1**(11), 763-766.

Ivanov, K. E. (1981). Water movements in minerals. Academic Press, London.

Johnson, L. C., & A.W. Damman. (1991). Species-controlled Sphagnum decay on a south Swedish raised bog. *Oikos*. **61**: 234-242.

Johnson, L. C., & Damman, A. W. H. (1993). Decay and its regulation in Sphagnum peatlands. *Advances in bryology*, **5**: 249-296.

Kadlec, R. H. (2009). Wastewater treatment at the Houghton Lake wetland: Hydrology and water quality. *Ecological Engineering*, **35**, 1287–1311. doi:10.1016/j.ecoleng.2008.10.001

Kadlec, R. H., & Wallace, S. D. (2009). Treatment Wetlands (2nd ed.). Boca Raton, FL: CRC Press, Taylor & Francis Group.

Kälviäinen, E. & Karunen, P. (1984). On the growth, senescence, and decay-resistance of Sphagnum mosses. Proc. 7th Int. Peat Congr. Dublin. **4**, 263-275.

Kimmel, K. & Mander, Ü. (2010). Ecosystem services of peatlands: Implications for restoration. *Progress in Physical Geography*, **34**(4): 491-514.

Kristjansson, J.K., P. Schonheit, & R.K. Thauer. (1982). Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate. *Archives fur Microbiology,* **131**, 278-282.

Lamers, L. P., Bobbink, R., & Roelofs, J. G. (2000). Natural nitrogen filter fails in polluted raised bogs. *Global Change Biology*, **6**(5), 583-586.

Larmola, T., Bubier, J. L., Kobyljanec, C., Basiliko, N., Juutinen, S., Humphreys, E., ... & Moore, T. R. (2013). Vegetation feedbacks of nutrient addition lead to a weaker carbon sink in an ombrotrophic bog. *Global change biology*, **19**(12): 3729-3739.

Lee, S. M., Jung, J. Y., & Chung, Y. C. (2000). Measurement of ammonia inhibition of microbial activity in biological wastewater treatment process using dehydrogenase assay. *Biotechnology letters*, **22**(12), 991-994.

Limpens, J., & Berendse, F. (2003). How litter quality affects mass loss and N loss from decomposing Sphagnum. *Oikos*, **103**(3), 537-547.

Lai, D.Y.F. (2009). Methane dynamics in Northern Peatlands: a review. *Pedosphere*, **19**, 409-421.

McCarter, C.P.R. (2016). The hydrochemical fate and transport of treated domestic wastewater contaminants during a wastewater polishing experiment in a sub-arctic ladder fen peatland. Ph.D. thesis, Geography Department, University of Waterloo, Waterloo, Ontario, Canada. *UWSpace. http://hdl.handle.net/10012/10937*

McCarter, C. P. R., Branfireun, B. A., & Price, J. S. (2017). Nutrient and mercury transport in a sub-arctic ladder fen peatland subjected to simulated wastewater discharges. *Science of The Total Environment*, ***609***, 1349-1360.

McCarter, C. P., & Price, J. S. (2017). Experimental hydrological forcing to illustrate water flow processes of a subarctic ladder fen peatland. *Hydrological Processes*.

Moore, T. R., & Dalva, M. (1993). The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. *Journal of Soil Science*, **44**(4), 651-664.

Moore, T. R. & Dalva, M. (1997). Methane and carbon dioxide exchange potentials of peat soils in aerobic and anaerobic laboratory incubations. *Soil Biology and Biochemistry*, **29**(8): 1157-1164.

Moore, T. R., Trofymow, J. A., Prescott, C. E., Titus, B. D., & CIDET Working Group. (2011). Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. *Plant and Soil*, **339**(1-2), 163-175.

Nilsson, M. & Bohlin, E. (1993). Methane and carbon dioxide concentrations in bogs and fens--with special reference to the effects of the botanical composition of the peat. *Journal of Ecology*, 615-625.

Parchomchuk, P., Neilsen, G. H., & Hogue, E. J. (1993). Effects of drip fertigation of NH4-N and P on soil pH and cation leaching. *Canadian Journal of Soil Science*, **73**(2), 157-164.

Preston, M. D., Smemo, K. A., McLaughlin, J. W., & Basiliko, N. (2012). Peatland microbial communities and decomposition processes in the James Bay Lowlands, Canada. *Frontiers in microbiology*, **3**, 70.

Puranen, R., Mäkilä, M., & Säävuori, H. (1999). Electric conductivity and temperature variations within a raised bog in Finland: implications for bog development. *The Holocene*, **9**(1), 13-24.

Riley, J.L. (2011). Wetlands of the Ontario Hudson Bay Lowland: An Ontario overview. Toronto, Ontario: Nature Conservancy of Canada.

Ronkanen, A.K. & Klove, B. (2009). Long-term phosphorus and nitrogen removal processes and preferential flow paths in northern constructed peatlands. *Ecological Engineering*, **35**. doi:10.1016/j.ecoleng.2008.12.007

Roulet, N. T. (2000). Peatlands, carbon storage, greenhouse gases, and the Kyoto protocol: prospects and significance for Canada. *Wetlands*, **20**(4), 605-615.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., ... & Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME journal*, **4**(10), 1340-1351.

Rydin, H., & Jeglum, J. K. (2013). The biology of peatlands, 2e. Oxford university press

Singh, J. S. & Gupta, S. R. (1977). Plant decomposition and soil respiration in terrestrial ecosystems. *The botanical review*, **43**(4): 449-528.

Sjörs, H. (1959). Bogs and fens in the Hudson Bay Lowlands. *Arctic*, **12**(1): 2-19.

Thormann, M. N., Bayley, S. E., & Currah, R. S. (2004). Microcosm tests of the effects of temperature and microbial species number on the decomposition of *Carex aquatilis* and *Sphagnum fuscum* litter from southern boreal peatlands. *Canadian journal of microbiology*, **50**(10): 793-802.

Turetsky, M. R., Treat, C. C., Waldrop, M. P., Waddington, J. M., Harden, J. W., & McGuire, A. D. (2008). Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *Journal of Geophysical Research*, **113**(G3).

Van Wijk, M. T., Clemmensen, K. E., Shaver, G. R., Williams, M., Callaghan, T. V., Chapin, F. S., ... & Lee, J. A. (2004). Long-term ecosystem level experiments at Toolik Lake, Alaska, and at Abisko, Northern Sweden: Generalizations and differences in ecosystem and plant type responses to global change. *Global Change Biology*, **10**(1), 105-123.

Wang, M., Moore, T. R., Talbot, J., & Richard, P. J. (2014). The cascade of C: N: P stoichiometry in an ombrotrophic peatland: from plants to peat. *Environmental Research Letters*, **9**(2), 024003.

Williams, B. L., & Silcock, D. J. (1997). Nutrient and microbial changes in the peat profile beneath Sphagnum magellanicum in response to additions of ammonium nitrate. *Journal of Applied Ecology*, 961-970.

Yavitt, J. B., Lang, G. E., & Wieder, R. K. (1987). Control of carbon mineralization to CH 4 and CO 2 in anaerobic, Sphagnum-derived peat from Big Run Bog, West Virginia. *Biogeochemistry*, **4**(2), 141-157.

Yavitt, J. B., Williams, C. J., & Wieder, R. K. (1997). Production of methane and carbon dioxide in peatland ecosystems across North America: effects of temperature, aeration, and organic chemistry of peat. *Geomicrobiology Journal*, **14**(4), 299-316.

Table 1. Separate analyses of variance by *Sphagnum* species of 40 day anaerobic CO_2 release, 24 hour aerobic CO_2 release and 40 day anaerobic CH_4 release during the incubation experiment as a function of *Sphagnum* origin, nutrient amendment and their interaction. Analyses were based on log-transformed data. Results with type I error level < 5% are shown in bold.

			40 day anaerobic CO_2			24 hr aerobic CO_2			40 day anaerobic CH_4		
Species	Source of variation	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Sphagnum fuscum	origin	2	0.238	11.5	**0.0002**	0.021	2.6	0.088	0.206	2.7	0.083
	nutrients	4	0.005	0.2	0.907	0.074	9.4	**<0.0001**	0.305	4.0	**0.010**
	origin x nutrients	8	0.018	0.9	0.552	0.027	3.4	**0.006**	0.057	0.7	0.650
	error	30	0.021			0.008			0.076		
Sphagum rubellum	origin	2	0.233	12.0	**0.0002**	0.815	88.5	**<0.0001**	19.113	115.0	**<0.0001**
	nutrients	4	0.021	1.1	0.393	0.015	1.6	0.189	0.877	5.3	**0.002**
	origin x nutrients	8	0.009	0.4	0.885	0.020	2.2	0.058	0.515	3.1	**0.011**
	error	30	0.020			0.009			0.166		
Sphagnum majus	origin	2	0.012	0.6	0.577	0.449	25.9	**<0.0001**	9.453	12.0	**0.0002**
	nutrients	4	0.030	1.4	0.271	0.038	2.2	0.092	0.770	1.0	0.436
	origin x nutrients	8	0.020	0.9	0.522	0.018	1.1	0.418	0.251	0.3	0.953
	error	30	0.022			0.017			0.790		

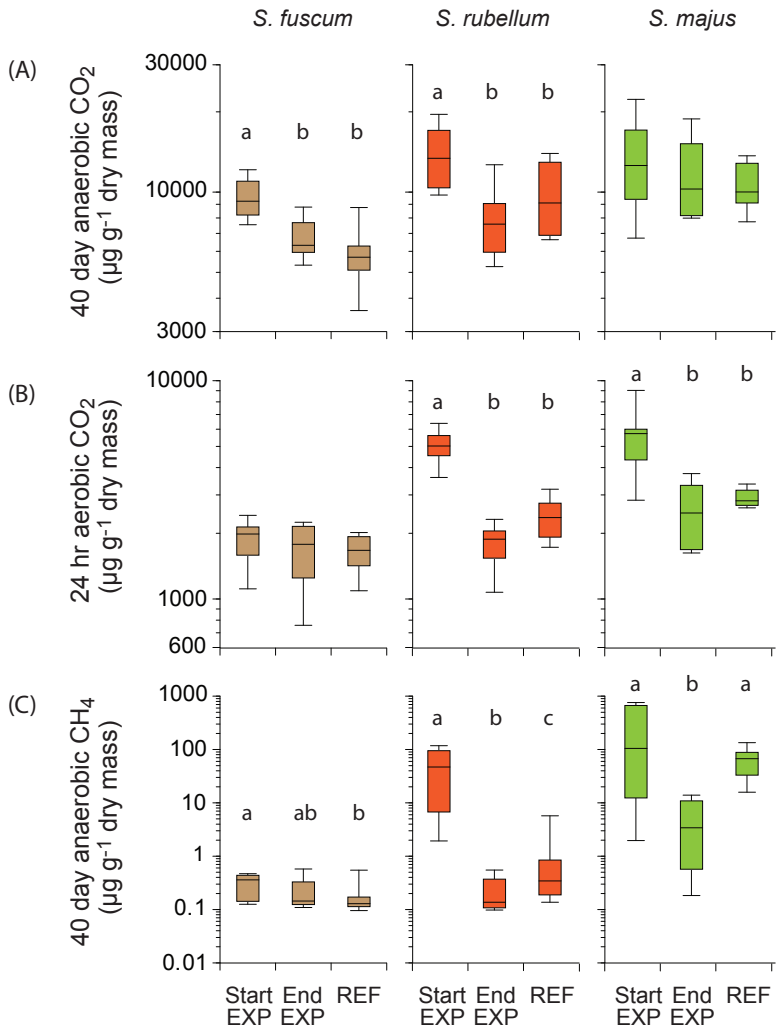

Figure 1. Box plots of the (A) anaerobic 40 day CO_2 production, (B) 24 hour aerobic CO_2 production, and (C) the 40 day anaerobic CH_4 production during the incubation of peat from *Sphagnum fuscum* (brown), *S. rubellum* (red) and *S. majus* (green) collected at the start and the end of the experimental fen and at the reference fen, across all nutrient amendment treatments. Letters above box plots show results of post-hoc Tukey test with a 5%Type I error rate, plots with no letters indicates no significant differences across peat origin.

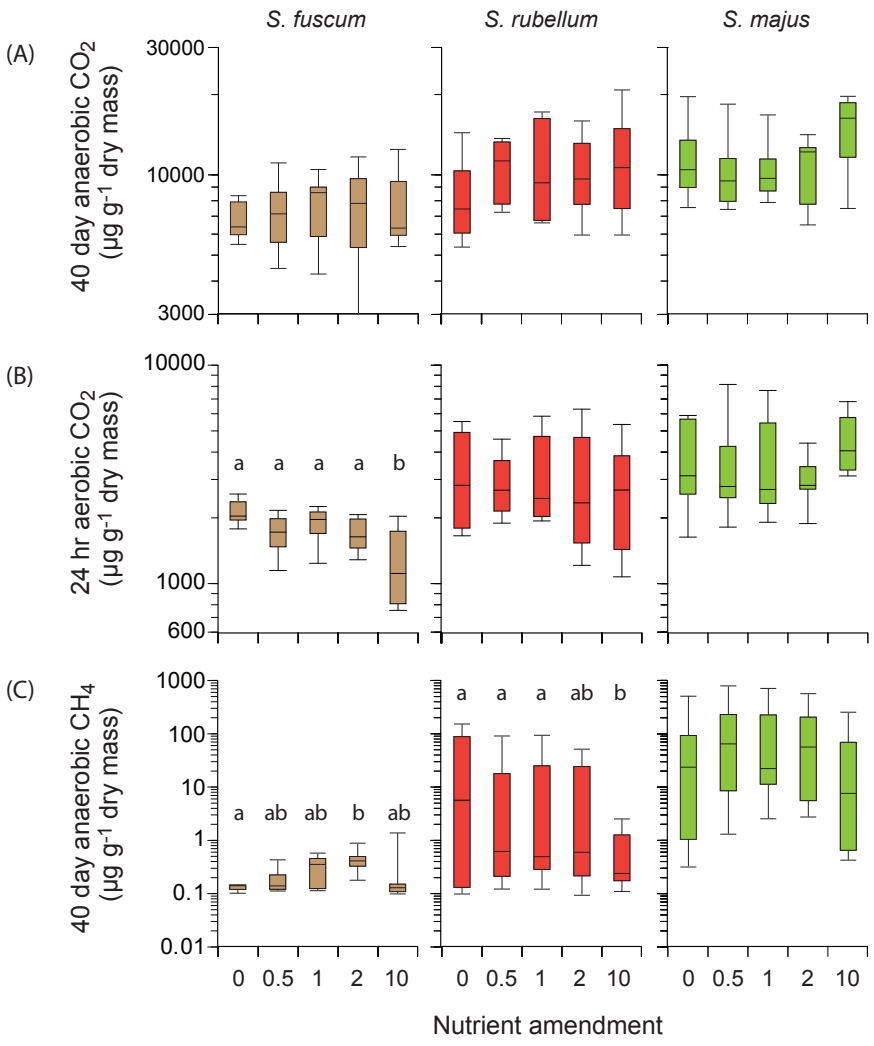

Figure 2. Box plots of the (A) anaerobic 40 day CO_2 production, (B) 24 hour aerobic CO_2 production, and (C) the 40 day anaerobic CH_4 production during the incubation of peat from *Sphagnum fuscum* (brown), *S. rubellum* (red) and *S. majus* (green) as a function of nutrient amendment levels ranging from zero (no amendment) to up to 10x the field rate of nutrient amendment. Letters above box plots show results of post-hoc Tukey test with a 5%Type I error rate, plots with no letters indicates no significant differences across nutrient amendment.

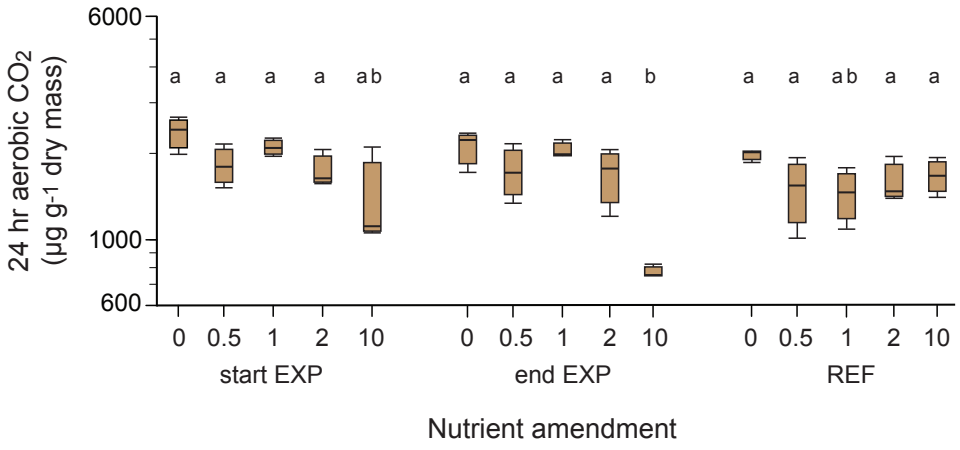

Figure 3. Box plots of the CO_2 produced after 24 hours under aerobic conditions for *S. fuscum* at each nutrient amendment level, ranging from zero (no amendment) to up to 10x the field rate of nutrient amendment, at all three peat origins. Letters above box plots refer to similar groups based on post hoc Tukey test with a 5%Type I error rate.

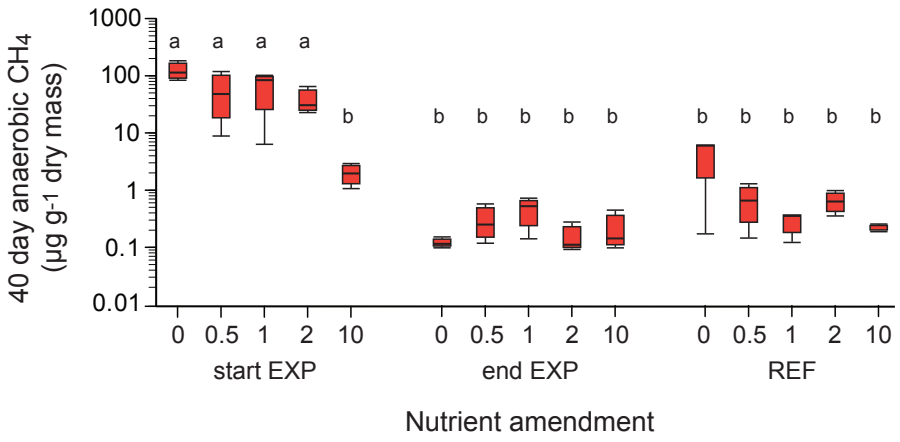

Figure 4. Box plots of the CH_4 produced after 40 days under anaerobic conditions for *S. rubellum* at each nutrient amendment level, ranging from zero (no amendment) to up to 10x the field rate of nutrient amendment, at all three peat origins. Letters above box plots refer to similar groups based on post hoc Tukey test with a 5%Type I error rate.

Research Implications

Sphagnum is the keystone genus within northern subarctic peatlands. Its health is closely tied to the quality and quantity of ecosystem services that these peatlands can provide. Our field-based research (chapter 1) found that short-term additions of simulated secondarily-treated wastewater to an experimental ribbed fen increased the primary productivity of *Sphagnum,* while decay rates remained low and comparable to an unamended ribbed reference fen. This implies that wastewater polishing will produce greater peat formation and greater carbon sequestration over the short term in these ribbed fens. Our incubation study (chapter 2), found that changes in quality and nutrient content of *Sphagnum* litter brought on during wastewater polishing play a larger role in its decay potential than the addition of further nutrients during the decomposition process. Ultimately, *Sphagnum* litter chemistry variables, possibly polyphenols: nutrient ratios, and C: nutrient ratios, appear to be the primary variables controlling microbial decomposition of *Sphagnum* moss.

Changes *Sphagnum* productivity and decomposition found in this study must be considered in light of the in hydrology, nutrient transport and geochemistry. McCarter *et al*. (2017) determined that the experimental ribbed fen at Victor Mine was highly effective at removing the added nutrients from the pore water as none of the added nutrient contaminants were detected at the outflow of the fen. The nutrients most common to domestic wastewater (NO_3, NH_4, and PO_4) remained effectively immobilized within the first or second peat ridge, and the SO_4^{2-} plume, although transported further downgradient than the other nutrient contaminants, was still effectively polished from the pore water, proving that the ribbed fen peatland hydrology and structure can be successful systems for wastewater polishing (McCarter *et al.*, 2017).

Further research on the biogeochemical interactions within the experimental fen at Victor Mine is ongoing especially in regard to methyl mercury (MeHg; L. Twible, Western University, in preparation). The ground water within the HBL region is rich in SO_4^{2-}, causing the domestic wastewater produced in the region to also be elevated in SO_4^{2-} concentrations (Steinback, 2012). Added SO_4^{2-} to waterlogged anoxic peat has been found elsewhere to lead to increased levels of MeHg, as the sulphate-reducing bacteria can facilitate methylation of mercury under these anaerobic conditions (Branfireun *et al*., 1999; Mitchell *et al*., 2008). Methyl mercury is a biological toxin, and once in the aquatic food web can bioaccumulate and biomagnify (Kidd *et al*., 1995). As this research on MeHg biogeochemistrybecomes available, it can be integrated with the hydrological results (McCarter and Price 2017; McCarter *et al.*, 2017) and *Sphagnum* growth and decomposition results (this study) to reach broader conclusions on the overall benefits of using ribbed fens to polish secondarily-treated wastewater.

Certainly, similar ribbed fens exist across boreal and subarctic Canada in the shield and in the HBL (Zoltai *et al.*, 1988; Riley, 2011). We only looked at one treatment ribbed fen, compared to one reference fen, but the studied fens appear to fairly represent other ribbed fens in the subarctic, in terms of their morphology and composition, if not in size. Given this similarity, we do not see how our *Sphagnum* productivity and decomposition results would not apply over the short term to other ribbed fens, if the nutrient and water inputs were scaled to the size of ribbed fens. Further study would be required to compare the hydrology and *Sphagnum* communities across a series of ribbed fens.

The experimental ribbed fen wetland at De Beers Victor Mine received simulated secondarily-treated domestic wastewater for two consecutive growing seasons (summer 2015 and 2016). We do not know what the medium or long term effects would be on *Sphagnum*

growth or productivity if fertilization were to continue. In theory, the additions of N and P to the peatland system would allow for vascular plants to obtain required nutrients to survive and grow. They will be able to produce taller shoots, thereby creating above-ground competition for light, out-shading the smaller plants (Moore *et al*., 1989; Wisheu and Keddy, 1992), including *Sphagnum* mosses. The taller plants will also produce more litter which will allow a secondary competitive effect over ground-dwelling plants (Facelli *et al*., 1991), such as *Sphagnum* mosses. Kadlec and Bevis (2009), found a strong shift to a tall and dense *Typha* dominated community after 30 years of nutrient addition in a more southern peatland which received secondarily treated wastewater. Based on these results from previous research, we predict that with longer-term nutrient addition, the plant community will shift away from *Sphagnum* dominance and move towards taller species such as tall graminoids and shrubs (Kadlec and Bevis, 2009; Bubier *et al*., 2007; Bragazza *et al*., 2004; Berendse *et al*., 2001). If vascular plants eventually out-shade the *Sphagnum*, any positive affect of increased *Sphagnum* productivity and increased peat formation would be temporary. The length of time that these subarctic ribbed fens can uptake nutrient without experiencing significant reduction to *Sphagnum* cover because of competition with taller vascular plants remains unknown.

To test this prediction of longer term effects of wastewater polishing in ribbed fens, the treatment fen would have to be continued, perhaps for as much as a decade. The establishment of many permanent vegetation sampling plots prior to any nutrient additions would also have been necessary. Only a few sampling plots were set up prior to the experiment, insufficient for any examination of changes in community assemblages over the short term. We can only comment on our visual observations of plant cover and diversity in summer 2015 verses summer 2016.

Both Dr. McCarter and I observe that the cover of graminoids appears as though it had increased within the first 50 meters down gradient from the point source nutrient load.

Again, longer-term hydrological and geochemical changes are also unclear. Kadlec (2009) found large shifts in the hydrology and nutrient relations of their treatment peatland over its 30-year lifespan. Further research would also be required on these aspects in subarctic ribbed fens.

The recovery time of these fens after the cessation of nutrient amendment is also unclear. Few studies have studied the addition of secondarily treated wastewater to peatlands. We know of no studies that examined the impacts on plant communities or ecosystem function after these waste water additions have been halted.

If all the short-term effects are sufficiently benign, one option to limit any (unknown) longer-term effects would be to move the outflow pipe into new polishing ribbed fens every few years. There are many unknowns with such a scenario, including the prospect of larger scale cumulative effects, requiring careful study, monitoring and management. Ultimately, the precautionary principle should apply.

There has been increasing pressure for industrial development on Ontario's far north (Far North Science Advisory Panel, 2010). If additional mines were to operate within the Hudson Bay Lowland, development planners will be required to have an environmentally responsible method for treating then polishing their domestic wastewater. The use of a natural wetland system to polish secondarily-treated wastewater, if selected carefully and well managed, could be more cost-effective relative to engineered tertiary treatment facilities. Ontario's far north is highly remote, transporting the good and materials needed for infrastructure is difficult, therefore developers may seek to reduce the amount of specialized infrastructure required for operation.

However, given the unknowns on the longer-term function of treatment ribbed fens, it is difficult to recommend the broader use of polishing peatlands, at least without further research.

This thesis suggests that the *Sphagnum* dominant nutrient-poor peatland within the HBL can successfully polish treated domestic wastewater over the short term, but there are still many uncertainties, some over the short term and many over the longer term. Research should be completed on a full range of potential environmental impacts associated with northern treatment wetlands and or polishing wetlands prior to recommending wider use of ribbed fens for wastewater polishing within the HBL. Until this research becomes available, it is difficult to consider in detail the implications, benefits, or impacts associated with the use of natural peatlands to polish wastewater in the far north.

Literature Cited

Berendse, F., Van Breemen, N., Rydin, H., Buttler, A., Heijmans, M., Hoosbeek, M. R., ... & Wallén, B. (2001). Raised atmospheric CO2 levels and increased N deposition cause shifts in plant species composition and production in Sphagnum bogs. *Global Change Biology*, **7**(5), 591-598.

Bragazza, L., Tahvanainen, T., Kutnar, L., Rydin, H., Limpens, J., Hájek, M., ... & Iacumin, P. (2004). Nutritional constraints in ombrotrophic Sphagnum plants under increasing atmospheric nitrogen deposition in Europe. *New Phytologist*, **163**(3), 609-616.

Branfireun, B.A., Roulet, N.T., Kelly, C.A., Rudd, J.W.M., 1999. In situ sulphate stimulation of mercury methylation in a boreal peatland: toward a link between acid rain and methylmercury contamination in remote environments. *Global Biogeochemical Cycles* **13**, 743–750. http://dx.doi.org/10.1029/1999gb900033.

Bubier, J. L., Moore, T. R., & Bledzki, L. A. (2007). Effects of nutrient addition on vegetation and carbon cycling in an ombrotrophic bog. *Global Change Biology*, **13**(6), 1168-1186.

Facelli, J.M. and Pickett, S.T.A. 1991. Plant litter: its dynamics and effects on plant community structure. The Botanical Review **57**: 1-32.

Far North Science Advisory Panel. (2010). Science for a Changing Far North. The Report of the Far North Science Advisory Panel. A report submitted to the Ontario Ministry of Natural Resources.

Kadlec, R. H. (2009). Wastewater treatment at the Houghton Lake wetland: Hydrology and water quality. *Ecological Engineering*, **35**, 1287–1311. doi:10.1016/j.ecoleng.2008.10.001

Kadlec, R. H., & F. B. Bevis. (2009). Wastewater treatment at the Houghton Lake wetland: Vegetation response. *Ecological Engineering*, **35**(9), 1312-1332.

Kidd, K.A., Hesslein, R.H., Fudge, R.J.P., and Hallard, K.A. (1995). The influence of trophic level as measured by d15N on mercury concentrations in fresh water organisms. *Water, Air, & Soil Pollution*, **80**, 1011–1015.

McCarter, C. P., & Price, J. S. (2017). The transport dynamics of chloride and sodium in a ladder fen during a continuous wastewater polishing experiment. *Journal of Hydrology*. **549**, 558-570.

McCarter, C. P. R., Branfireun, B. A., & Price, J. S. (2017). Nutrient and mercury transport in a sub-arctic ladder fen peatland subjected to simulated wastewater discharges. *Science of The Total Environment*, ***609***, 1349-1360.

Mitchell, C.P.J., Branfireun, B.A., Kolka, R.K., (2008). Assessing sulfate and carbon controls on net methylmercury production in peatlands: an in situ mesocosm approach. *Applied Geochemistry*, **23**, 503–518. http://dx.doi.org/10.1016/j.apgeochem.2007.12.020.

Moore, D.R.J., Keddy, P.A., Gaudet, C.L. and Wisheu, I.C. 1989. Conservation of wetlands: do infertile wetlands deserve a higher priority? Biological Conservation **47**: 203-217.

Riley, J.L. 2011. Wetlands of the Hudson Bay Lowland: An Ontario Overview. Nature Conservancy of Canada, Toronto. 156 pp.

Steinback, B., 2012. De Beers Canada Inc. Victor Mine Northeast Fen 2011 Annual Report Per Condition 8(3) of Certificate of Approval no. 4056-6w8qbu. De Beers Canada Inc., Victor Mine, Ontario, Canada, pp. 1–31.

Wisheu, I.C. and Keddy, P.A. 1992. Competition and centrifugal organization of plant communities: theory and tests. Journal of Vegetation Science **3**: 147-156.

Zoltai, S.C., Taylor, S.J., Jeglum, J.K., Mills, G.F. and Johnson, J.D. 1988. Wetlands of boreal Canada. Pages 97-154 *in* N. W. W. Group, ed. Wetlands of Canada. Polyscience Publications, Montreal. http://www.cfs.nrcan.gc.ca/pubwarehouse/pdfs/19220.pdf

Appendix

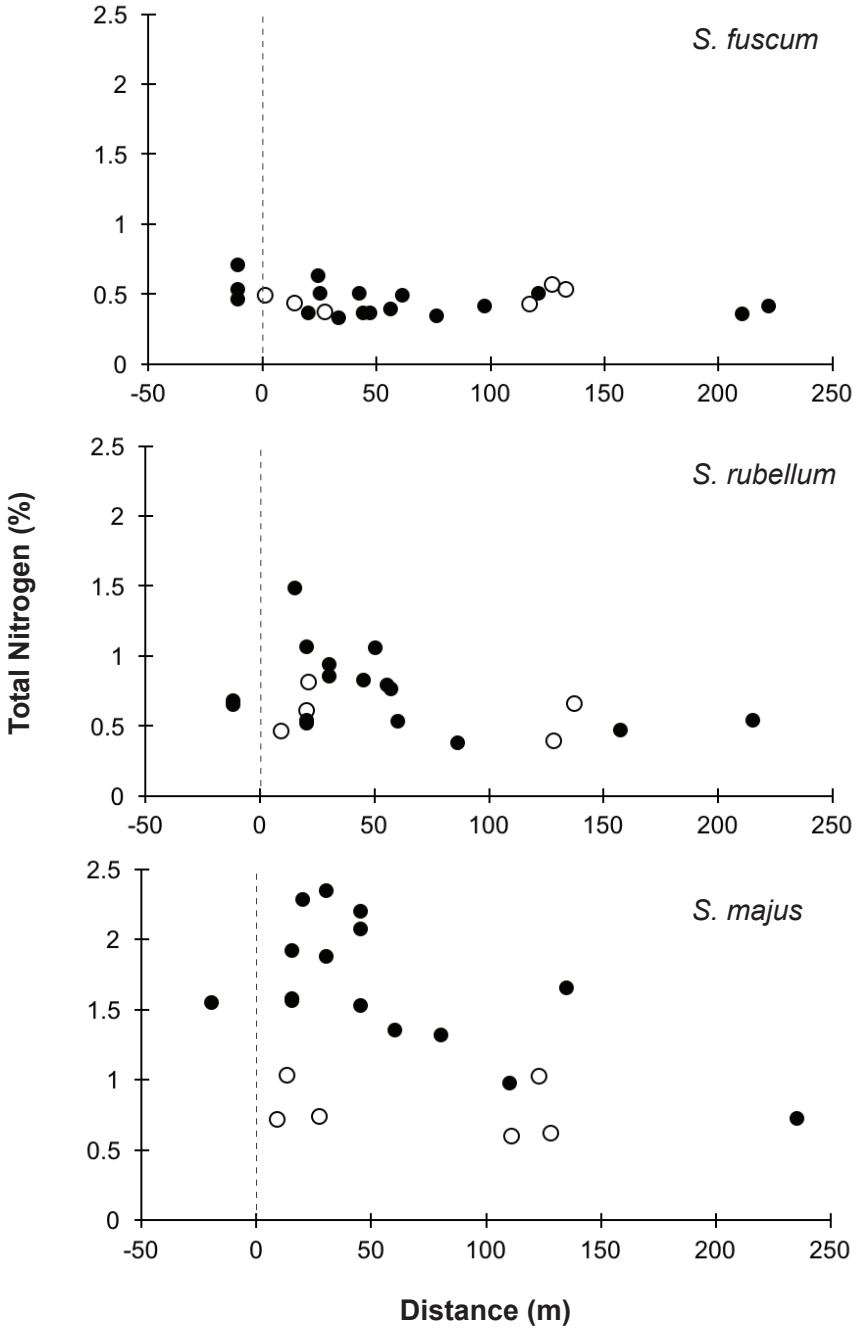

Figure A1. Scatter plot of Total Nitrogen content (percent mass) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

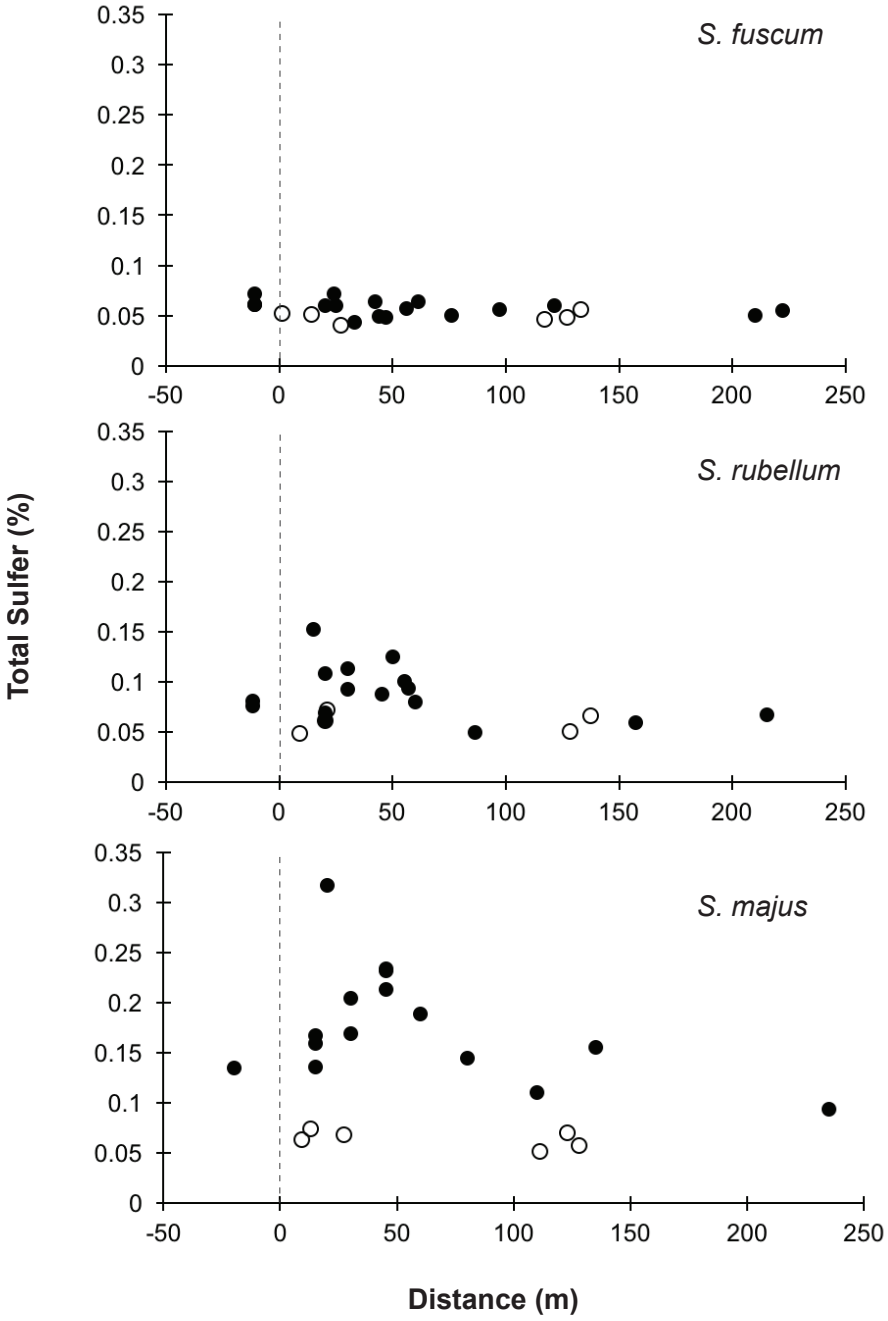

Figure 2A. Scatter plot of Total Sulfer content (percent mass) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

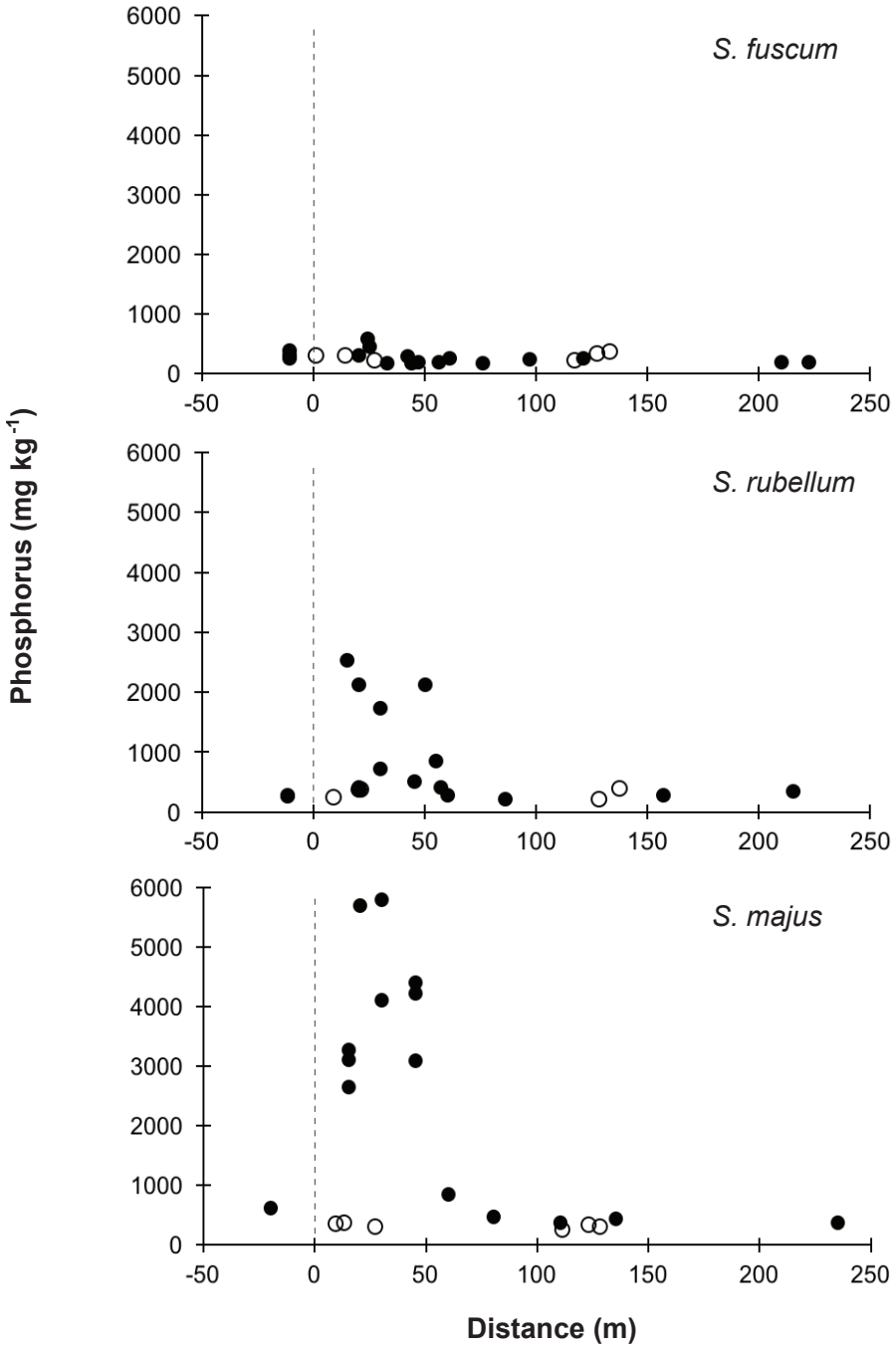

Figure 3A. Scatter plot of Phosphorus concentration (mg kg^{-1}) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

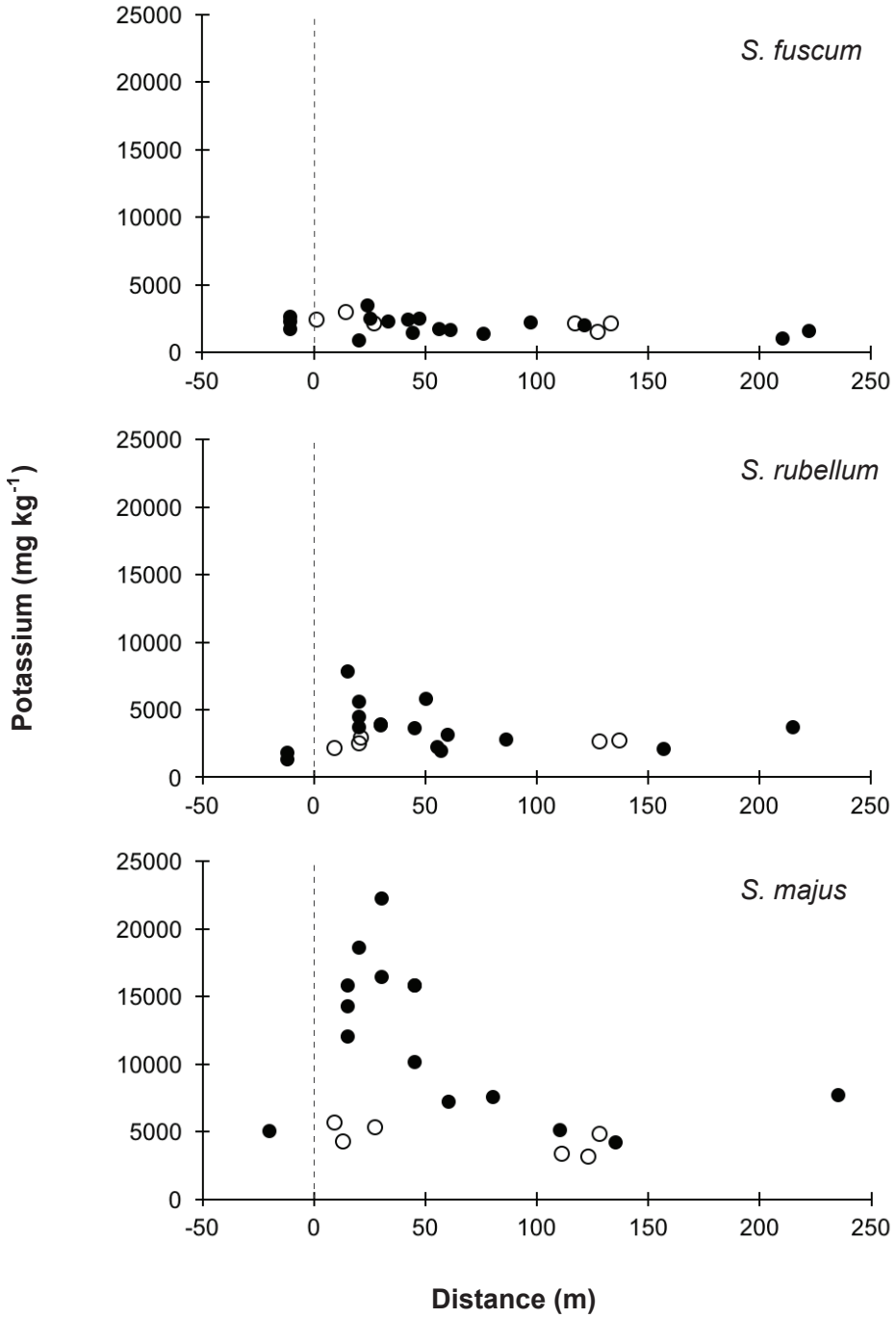

Figure 4A. Scatter plot of Potassium concentration (mg kg^{-1}) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

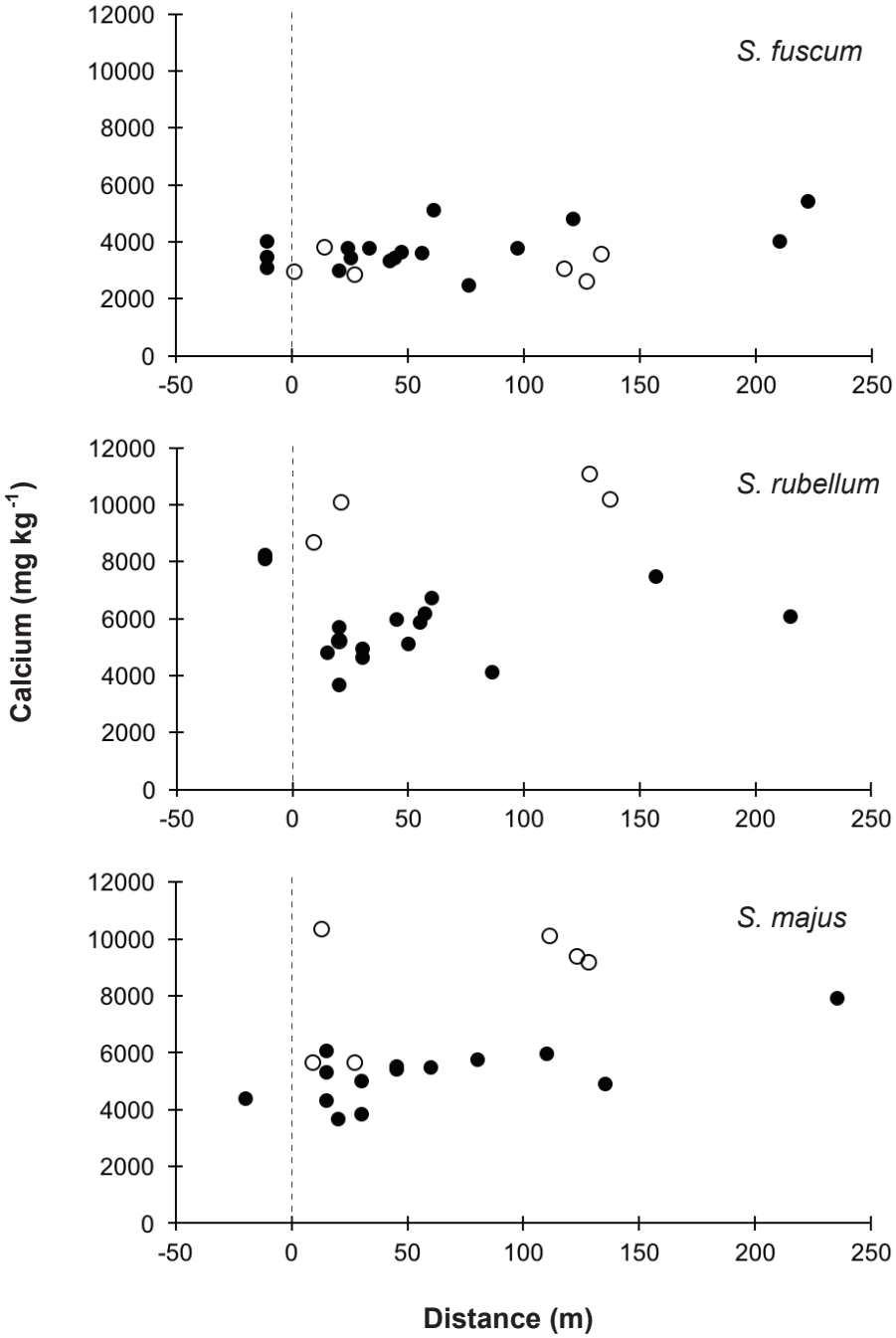

Figure 5A. Scatter plot of Calcium concentration (mg kg^{-1}) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.

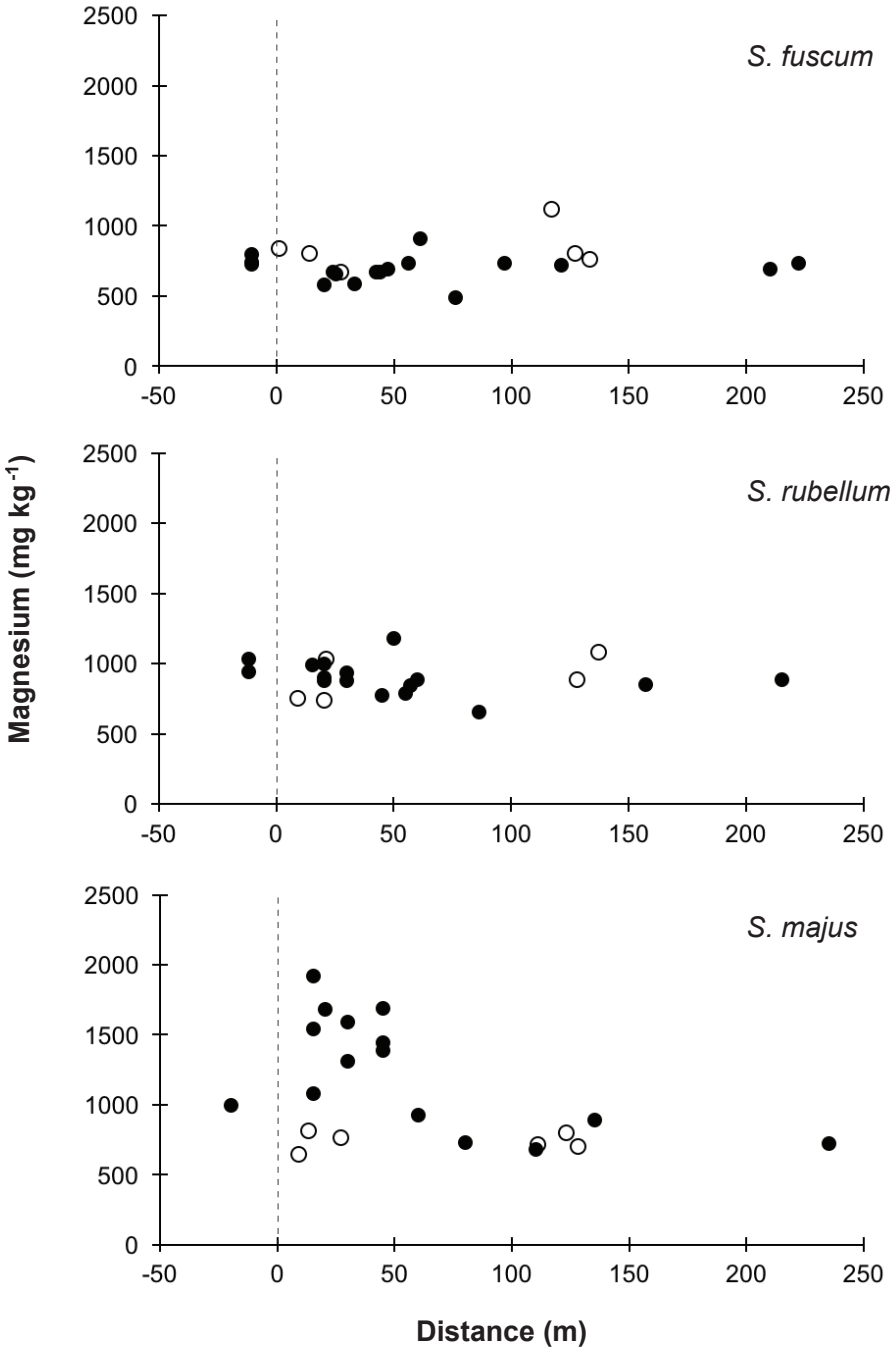

Figure 6A. Scatter plot of Magnesium concentration (mg kg^{-1}) within *Sphagnum fuscum* (top), *Sphagnum reubellum* (centre), and *Sphagnum majus* (bottom) over distance (m), experimental fen (solid black circles) and the reference fen (open circles). The vertical dashed line marks the location of the discharge point of nutrients, and negative distance values correspond to sample sites up gradient from the point source.